বাংলাদেশ স্ট্যান্ডার্ডস এন্ড টেস্টিং ইনস্টিটিউশন
শিল্প মন্ত্রণালয়
গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
মান ভবন, ১১৬/ক, তেজগাঁও শিল্প এলাকা, ঢাকা-১২০৮।
ফোন: ৮৮৭০২৭৫, ৮৮৭০২৭৭, ৮৮৭০২৭৮, ৮৮৭০২৭৯, ৮৮৭০২৮০, ৮৮৭০২৮১, ৯১৩১৫৮২
Fax : 88-02-9131581, E-mail : dg@bsti.gov.bd, bsti@bangla.net, , Website : www.bsti.gov.bd

মুজিববর্ষের দর্শন
টেকসই শিল্পায়ন।

Research Completion Report

Research Proposal:

Development of an improved method in BSTI for analysis of Copper in Bakery products by Atomic Absorption Spectrophotometer(AAS)

Submitted by: Mst. Nazneen Akter, Assistant Director (Chemical) and
Dr. Md. Shahed Reza, Assistant Director (Chemical)

Bangladesh Standards and Testing Institution (BSTI)

Duration: 14.03.2021 to 15.06.2022

Table of Content

Serial Number	Title	Description	Page Number
01	Research Proposal	Development of an improved method in BSTI for analysis of Copper in Bakery products by Atomic Absorption Spectrophotometer(AAS)	3-4
02	Method Validation Protocol	Method validation performance characteristics	5-6
03	Method Validation Report	Method validation report for determination of Copper in Biscuit by Atomic Absorption Spectrophotometer (AAS)	7-18
04	Discussion and Conclusion	Discussion and Conclusion about the newly developed improved method	19

Research Proposal

Sl. No. & Title	Description
1. Title of the Research	Development of an improved method in BSTI for analysis of Copper in Bakery products by Atomic Absorption Spectrophotometer(AAS)
2. Research Problem	In recent years, copper (Cu) enters in agricultural soils, due to arbitrary use of pesticides, fungicides, industrial effluent and wastewater irrigation now it is a major concern for sustainable food production especially in developing countries. Copper enters in food system from environment. There are few research work done in this topic. Therefore, it is necessary to develop an improve method to determine Copper in Bakery products accurately in the BSTI laboratory.
3. Justification	Copper toxicity is a type of metal poisoning caused by an excess of copper in the body. Acute symptoms of copper poisoning by ingestion include vomiting, hematemesis, hypotension, melena (black "tarry" feces), jaundice (yellowish pigmentation of the skin), and gastrointestinal distress. Bangladesh Food Safety Authority (BFSA) published a regulation regarding food contaminant in 2017. BFSA set the maximum limit of Copper for Bakery products. So it is very important to analyze Copper accurately in Bakery product.
4. Gap of Previous Research	Presently in BSTI Copper is not determined in Bakery product because it is not included in Bangladesh Standard Specification but there might be a chance of having Copper in Bakery products.
5. Audience	The Scientist, Laboratory analyst throughout the world will be the audience.
6. Questions	How BSTI can develop a method to be effective, easier, rapid and fit for purpose for analysis Copper in Bakery product?
7. Purpose	To develop an efficient method for analysis of Copper by AAS in BSTI Laboratory for Bakery products.
8. Title	Development of an improved method in BSTI for analysis of Copper in Bakery products by Atomic Absorption Spectrophotometer(AAS)
9. Methodology	Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions. The technique makes use of the atomic absorption spectrum of a sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert law. This validation is to prove that the method developed for the determination of Copper in Bakery product is suitable for its intended use "*fit for purpose*". Method Validation will be performed using Biscuit as a representative matrix.

10. Time Frame and Tentative Budget	The project needs 06 (Six) months time depending on financial and logistic support. It requires approximately Taka 1,50,000/- (One lac and fifty thousand) for sample collection, procurement of following reagent, chemicals, Certified Reference Materials, Spares of AAS, sample preparation accessories etc. a) Certified Reference Material of Copper- 40,000 (Forty Thousand only). b) Reagent and Chemical- 40,000 (Forty Thousand only). c) Consumables of AAS- 40,000 (Forty Thousand only). d) Contingency, Travel, Training, Stationary, etc. and others for research work tk. 30,000 (Thirty Thousand only). This is a tentative budget. Expenditure for each category may increase or decrease at purchase time (with constant total budget) .
11. Bibliography	Bibliography will be given at the end of research paper.

Method Validation Protocol

In this research at first the analytical methods were validated. The validation was performed as described in this study in line with international guideline Eurachem**.** After validation of methods the samples were analyzed using that validated method. The following method validation performance characteristics were performed in this study.

(a) Selectivity
(b) Limit of Detection (LOD)
(c) Limit of Quantification(LOQ)
(d) Working Range and Linearity
(e) Accuracy (Recovery)
(f) Precision (Repeatability)

Selectivity

Selectivity relates to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ was calculated using the following formula

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

Where,
S_0 is the estimated standard deviation of single results at or near zero concentration
$S_0'$is the standard deviation used for calculating LOD and LOQ.
n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.
n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD was calculated as 3×So′ and LOQ was calculated as 10×So′

Working Range and Linearity

The working range is an interval, in which a method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ.

The upper end of the working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed.

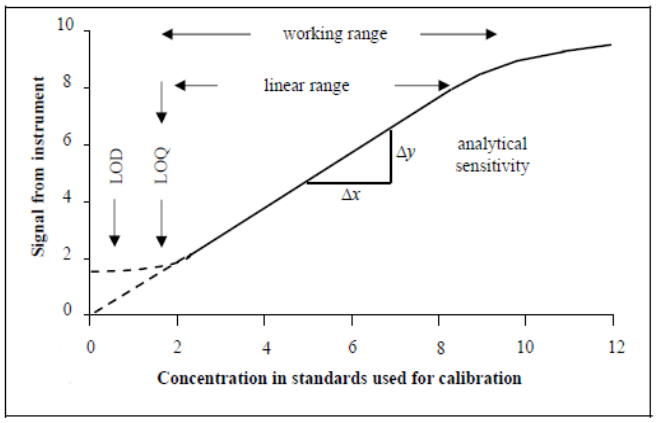


Figure 01: Analytical sensitivity, working range and linear range

The Figure-01 shows a response curve obtained with an instrumental method. The working range, linear range, analytical sensitivity, LOD and LOQ are identified**.**

Accuracy (Recovery)

Accuracy is the closeness of a single result to a reference value. Method validation need to investigate the accuracy of results by considering both systematic and random effects on single results.

Accuracy can be expressed as a relative recovery

$$R(\%) = \frac{x' - \bar{x}}{x_{spike}} \times 100$$

x′ = is the mean value of the spiked sample, $\bar{x}$ is the mean value of unspike sample and x_{spike} is the added concentration.

Precision (Repeatability)

Replication is essential for obtaining reliable estimation of precision. Replicate analysis are designed to take into consideration of all the variations in analytical conditions which is expected during routine use of the method. Precision is expressed as a relative standard deviation since it is approximately constant over the range of interest**.**

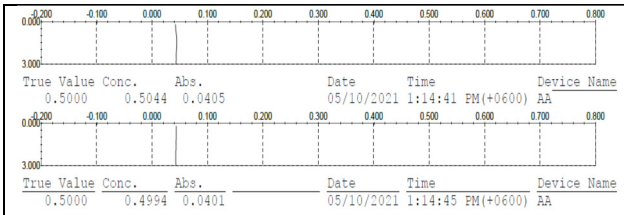
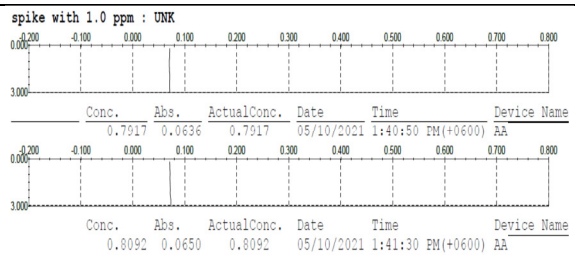
Relative Standard Deviation is calculated as RSD % = $\frac{SD}{Average} \times 100$

Method Validation Report

Method validation report for determination of Copper in Biscuit by Atomic Absorption Spectrophotometer (AAS)

Selectivity

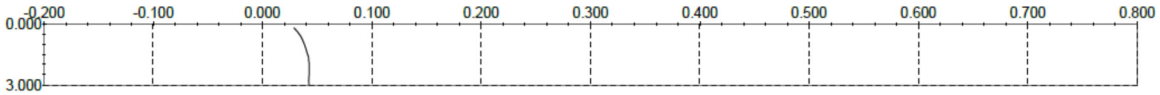
Analytical selectivity relates to "the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior" A absorbance in a absorption spectrum identified as being due to the analyte of interest on the basis that an Reference Material (RM) containing the analyte generates a signal at the same wavelength on the absorption spectrum.

	
Absorption spectrum of Certified Reference Material(CRM)of Copper at Concentration 0.5 mg/L	Absorption spectrum of Spiked Biscuit with CRM of Copper at Concentration 1.0 mg/L

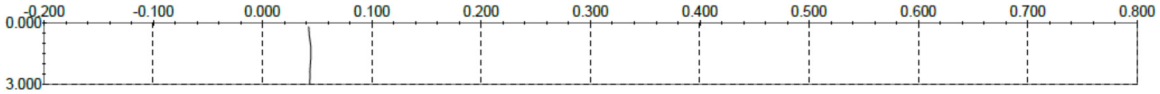
The above Absorption spectrums are the supportive evidence to demonstrate selectivity of Copper in Biscuit

Limit of Detection (LOD) and Limit of Quantification (LOQ)

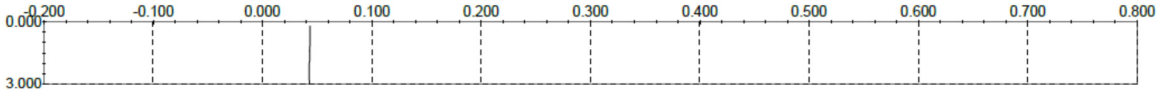
Std-2 : STD



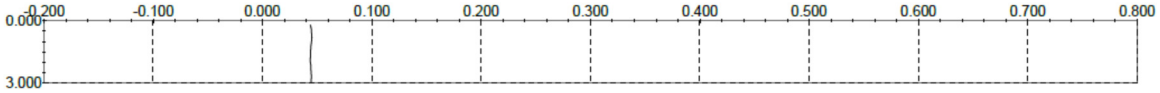
True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4521	0.0363	05/10/2021	1:14:01 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5044	0.0405	05/10/2021	1:14:41 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4994	0.0401	05/10/2021	1:14:45 PM(+0600)	AA

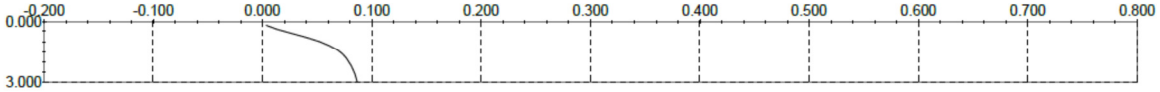


True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5143	0.0413	05/10/2021	1:14:49 PM(+0600)	AA

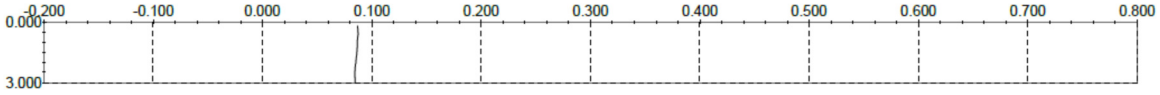
Std-2 : STD Average

True Value	Conc.	Abs.	%RSD	SD
0.5000	0.5056	0.0406	1.50	0.0006

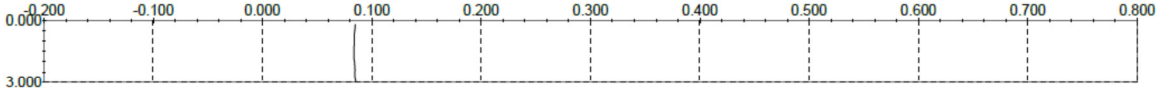
Std-3 : STD



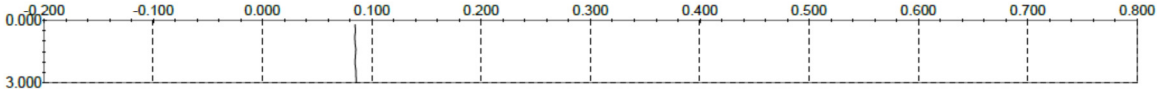
True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	0.7221	0.0580	05/10/2021	1:14:56 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0318	0.0829	05/10/2021	1:15:36 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0095	0.0811	05/10/2021	1:15:40 PM(+0600)	AA

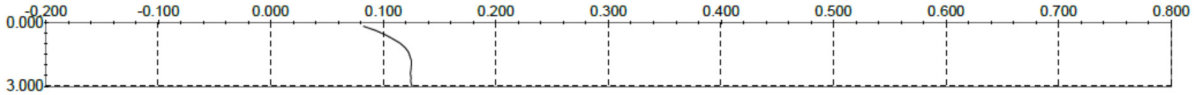


True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0194	0.0819	05/10/2021	1:15:43 PM(+0600)	AA

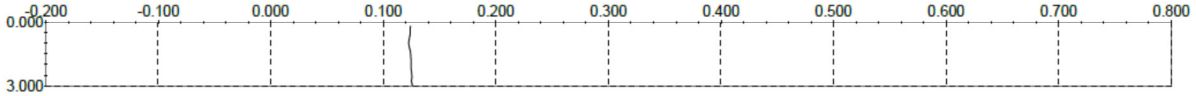
Std-3 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.0000	1.0207	0.0820	1.10	0.0009

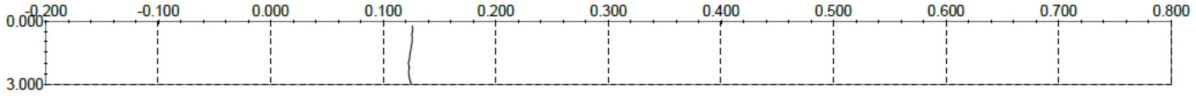
Std-4 : STD



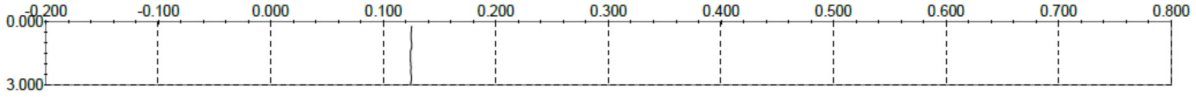
True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.4051	0.1129	05/10/2021	1:15:50 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:30 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:34 PM(+0600)	AA

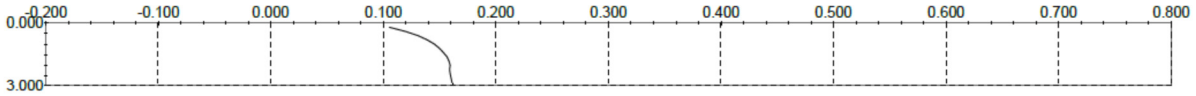


True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5121	0.1215	05/10/2021	1:16:38 PM(+0600)	AA

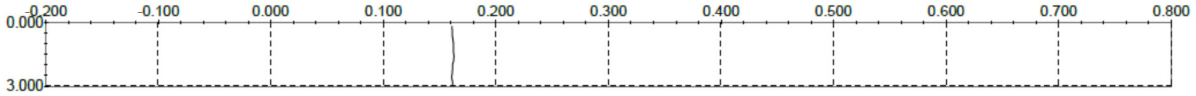
Std-4 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.5000	1.5096	0.1213	0.14	0.0002

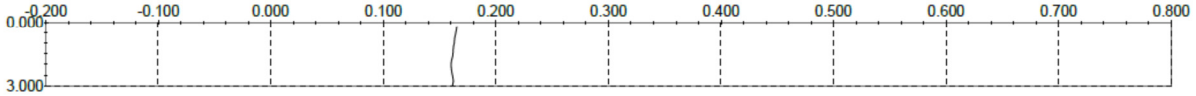
Std-5 : STD



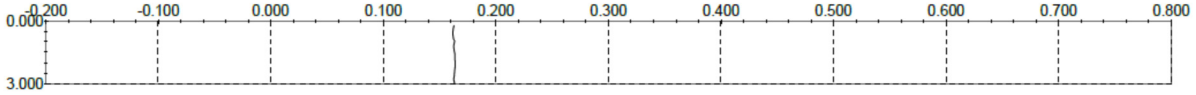
True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.8032	0.1449	05/10/2021	1:16:45 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9736	0.1586	05/10/2021	1:17:25 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9811	0.1592	05/10/2021	1:17:29 PM(+0600)	AA

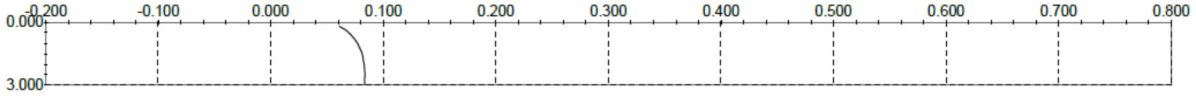


True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9898	0.1599	05/10/2021	1:17:33 PM(+0600)	AA

Std-5 : STD Average

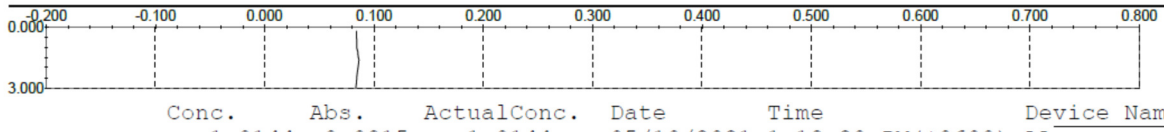
True Value	Conc.	Abs.	%RSD	SD
2.0000	1.9811	0.1592	0.41	0.0007

1.0ppm : UNK

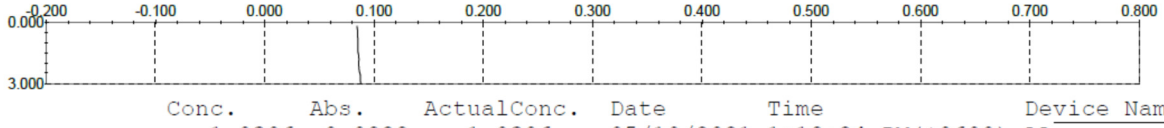


	Conc.	Abs.	ActualConc.	Date	Time	Device Name
	0.9361	0.0752	0.9361	05/10/2021	1:17:40 PM(+0600)	AA

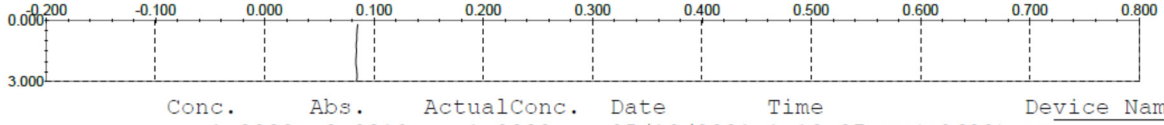
Cu 05 October 2021



Conc.	Abs.	ActualConc.	Date	Time	Device Name
1.0144	0.0815	1.0144	05/10/2021	1:18:20 PM(+0600)	AA



Conc.	Abs.	ActualConc.	Date	Time	Device Name
1.0306	0.0828	1.0306	05/10/2021	1:18:24 PM(+0600)	AA



Conc.	Abs.	ActualConc.	Date	Time	Device Name
1.0082	0.0810	1.0082	05/10/2021	1:18:27 PM(+0600)	AA

1.0ppm : UNK Average

Conc.	Abs.	ActualConc.	Actual Conc. Unit	%RSD	SD
1.0182	0.0818	1.0182	ppm	1.14	0.0009

We used the below formula for calculation of LOD and LOQ

$$s_0' = s_0 \sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

s_0 is the estimated standard deviation of m single results at or near zero concentration.

s_0' is the standard deviation used for calculating LOD and LOQ.

n is the number of replicate observations averaged when reporting results where each replicate is obtained following the entire measurement procedure.

n_b is the number of blank observations averaged when calculating the blank correction according to the measurement procedure.

LOD= 3×So´ and LOQ= 10×So´

For Copper in Biscuit

Sl. No	Abs	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	0.041	0.5034	0.5140	9.7934
2	0.0408	0.5009	0.5002	10.0135
3	0.0404	0.4959	0.5011	9.8956
4	0.0411	0.5046	0.5142	9.8139
5	0.0418	0.5134	0.5047	10.1723
6	0.0398	0.4884	0.5022	9.7243
7	0.0401	0.4921	0.5019	9.8050
8	0.0399	0.4896	0.5177	9.4573
9	0.0404	0.4959	0.5101	9.7210
10	0.0404	0.4959	0.5014	9.8897

So	1/No. Rep	1/No. Blk	0.1+0.1	SQRT of 0.2	So′	**LOD(mg/kg)**	**LOQ(mg/kg)**
0.1894	0.10	0.10	0.20	0.45	0.0847	**0.2541**	**0.8471**

Working Range and Linearity

Std-1 : STD

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

3.000

True Value	Conc.	Abs.	Date	Time	Device Name
0.0000	-0.0132	-0.0011	05/10/2021	1:13:07 PM(+0600)	AA

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

3.000

True Value	Conc.	Abs.	Date	Time	Device Name
0.0000	-0.0169	-0.0014	05/10/2021	1:13:47 PM(+0600)	AA

-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

3.000

True Value	Conc.	Abs.	Date	Time	Device Name
0.0000	-0.0169	-0.0014	05/10/2021	1:13:50 PM(+0600)	AA

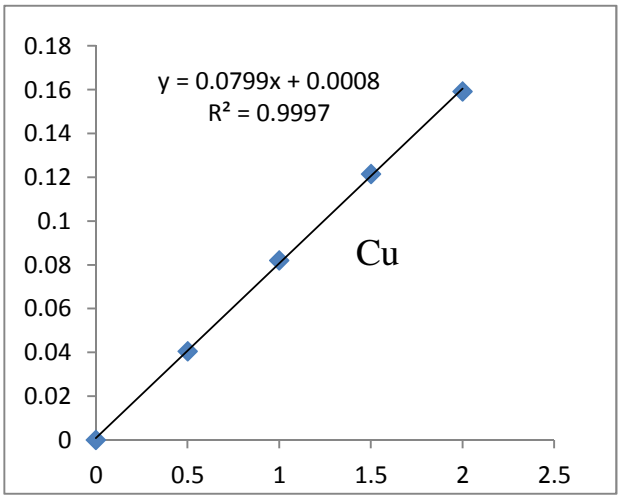
-0.200 -0.100 0.000 0.100 0.200 0.300 0.400 0.500 0.600 0.700 0.800

0.000

3.000

True Value	Conc.	Abs.	Date	Time	Device Name
0.0000	-0.0157	-0.0013	05/10/2021	1:13:54 PM(+0600)	AA

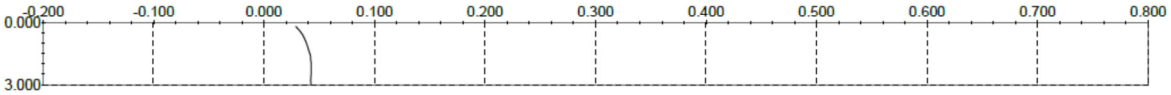
Conc(mg/L)	Absorbance
0	0
0.5	0.0406
1	0.082
1.5	0.1215
2	0.1592
Intercept	0.0008
Slope	0.0799



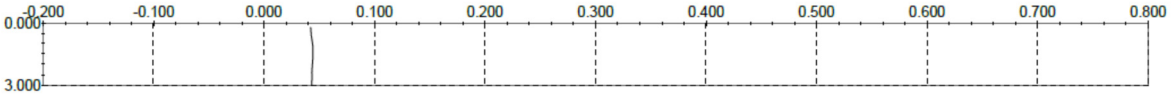
Std-1 : STD Average

True Value	Conc.	Abs.	%RSD	SD
0.0000	-0.0169	-0.0014	4.22	0.0001

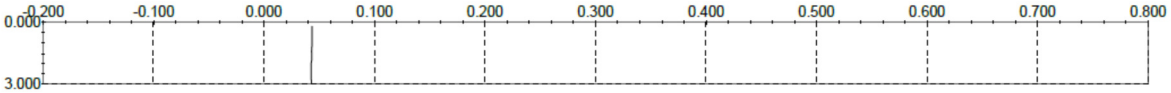
Std-2 : STD



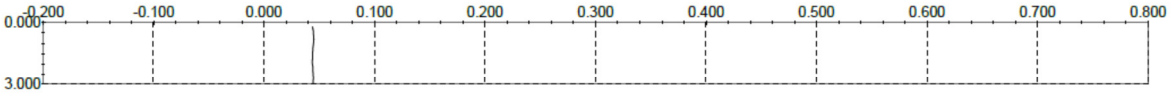
True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4521	0.0363	05/10/2021	1:14:01 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5044	0.0405	05/10/2021	1:14:41 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4994	0.0401	05/10/2021	1:14:45 PM(+0600)	AA

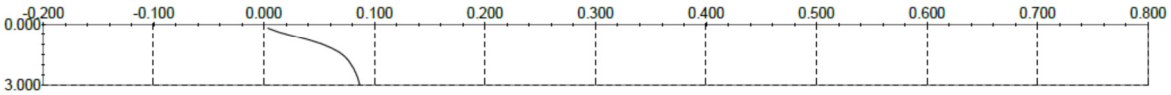


True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5143	0.0413	05/10/2021	1:14:49 PM(+0600)	AA

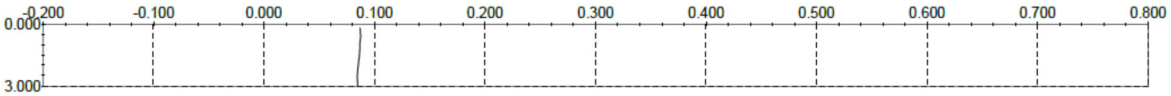
Std-2 : STD Average

True Value	Conc.	Abs.	%RSD	SD
0.5000	0.5056	0.0406	1.50	0.0006

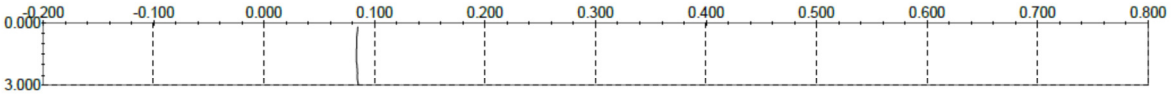
Std-3 : STD



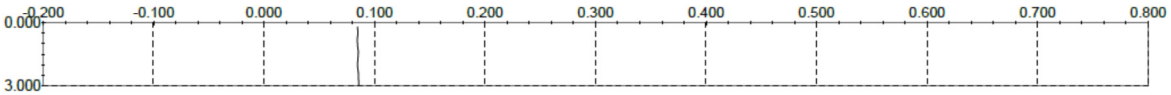
True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	0.7221	0.0580	05/10/2021	1:14:56 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0318	0.0829	05/10/2021	1:15:36 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0095	0.0811	05/10/2021	1:15:40 PM(+0600)	AA

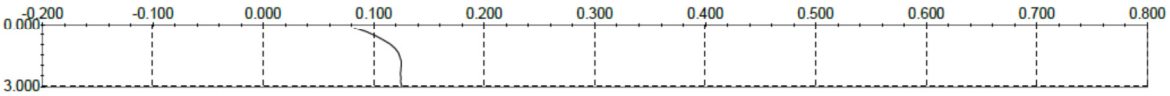


True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0194	0.0819	05/10/2021	1:15:43 PM(+0600)	AA

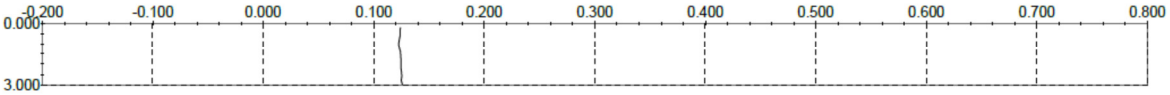
Std-3 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.0000	1.0207	0.0820	1.10	0.0009

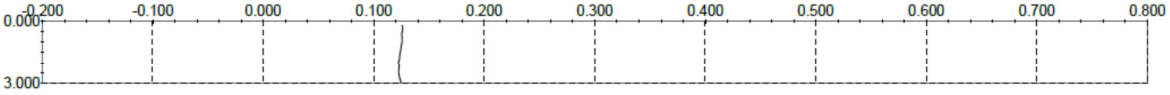
Std-4 : STD



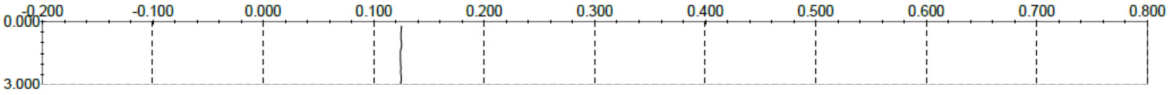
True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.4051	0.1129	05/10/2021	1:15:50 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:30 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:34 PM(+0600)	AA

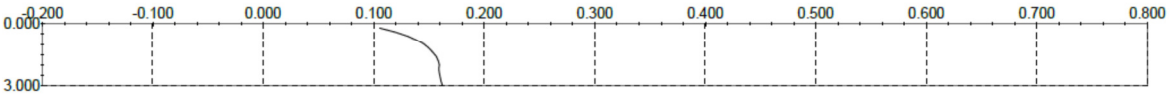


True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5121	0.1215	05/10/2021	1:16:38 PM(+0600)	AA

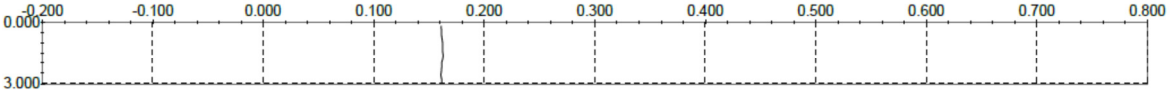
Std-4 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.5000	1.5096	0.1213	0.14	0.0002

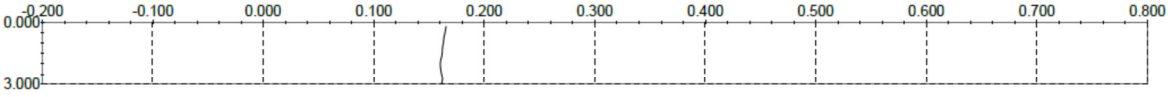
Std-5 : STD



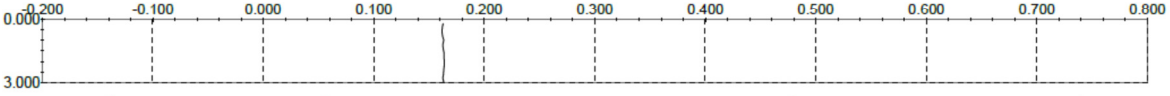
True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.8032	0.1449	05/10/2021	1:16:45 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9736	0.1586	05/10/2021	1:17:25 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9811	0.1592	05/10/2021	1:17:29 PM(+0600)	AA



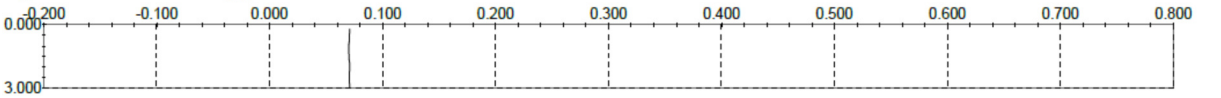
True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9898	0.1599	05/10/2021	1:17:33 PM(+0600)	AA

Std-5 : STD Average

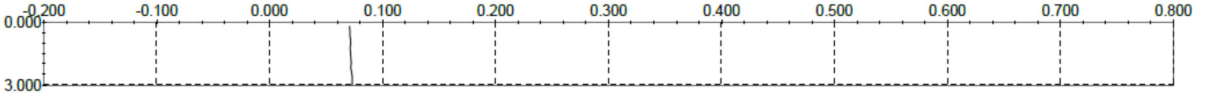
True Value	Conc.	Abs.	%RSD	SD
2.0000	1.9811	0.1592	0.41	0.0007

Trueness (Spike Recovery)

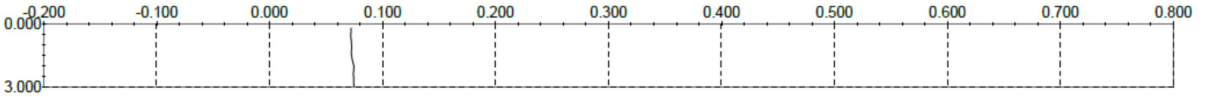
spike with 1.0 ppm : UNK



Conc.	Abs.	ActualConc.	Date	Time	Device Name
0.7917	0.0636	0.7917	05/10/2021	1:40:50 PM(+0600)	AA

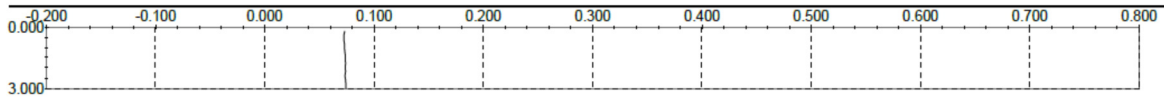


Conc.	Abs.	ActualConc.	Date	Time	Device Name
0.8092	0.0650	0.8092	05/10/2021	1:41:30 PM(+0600)	AA



Conc.	Abs.	ActualConc.	Date	Time	Device Name
0.8266	0.0664	0.8266	05/10/2021	1:41:34 PM(+0600)	AA

Cu 05 October 2021



Conc.	Abs.	ActualConc.	Date	Time	Device Name
0.8291	0.0666	0.8291	05/10/2021	1:41:38 PM(+0600)	AA

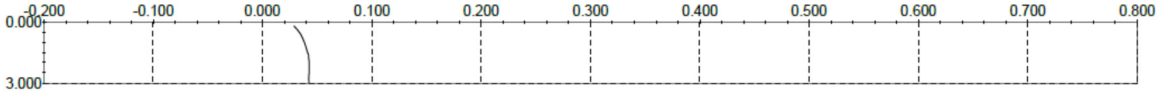
spike with 1.0 ppm : UNK Average

Conc.	Abs.	ActualConc.	Actual Conc. Unit	%RSD	SD
0.8216	0.0660	0.8216	ppm	1.32	0.0009

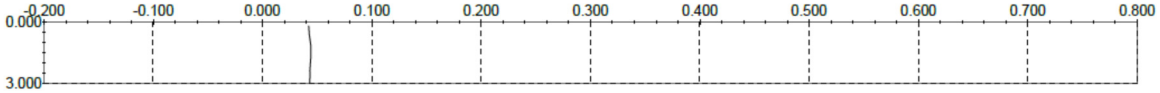
Sl. No	Abs	Conc(mg/L)	Recovery %
1	0.0666	0.8239	82.3942
2	0.0664	0.8214	82.1438
		Average	**82.27**

Precision (Repeatability)

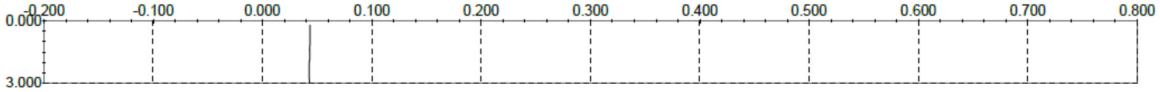
Std-2 : STD



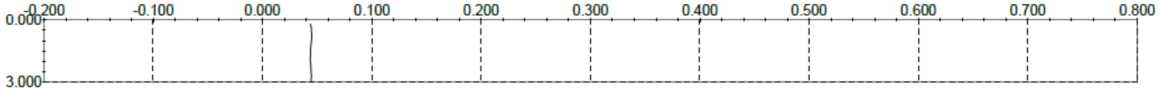
True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4521	0.0363	05/10/2021	1:14:01 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5044	0.0405	05/10/2021	1:14:41 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.4994	0.0401	05/10/2021	1:14:45 PM(+0600)	AA

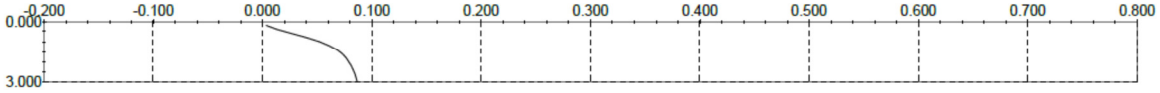


True Value	Conc.	Abs.	Date	Time	Device Name
0.5000	0.5143	0.0413	05/10/2021	1:14:49 PM(+0600)	AA

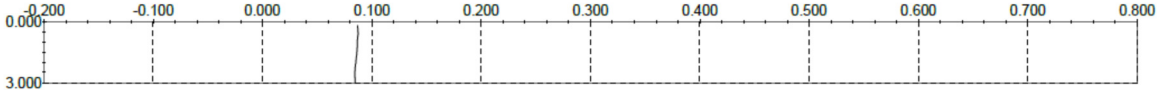
Std-2 : STD Average

True Value	Conc.	Abs.	%RSD	SD
0.5000	0.5056	0.0406	1.50	0.0006

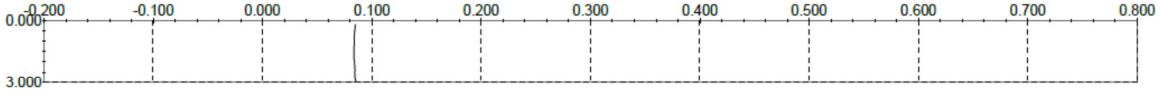
Std-3 : STD



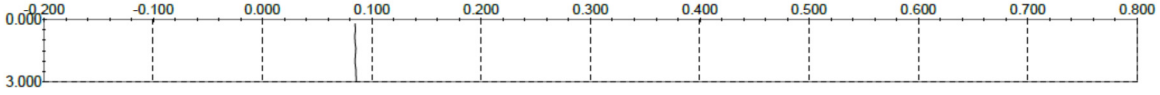
True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	0.7221	0.0580	05/10/2021	1:14:56 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0318	0.0829	05/10/2021	1:15:36 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0095	0.0811	05/10/2021	1:15:40 PM(+0600)	AA

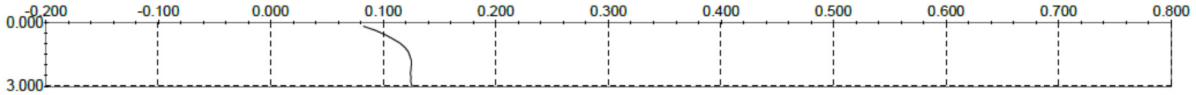


True Value	Conc.	Abs.	Date	Time	Device Name
1.0000	1.0194	0.0819	05/10/2021	1:15:43 PM(+0600)	AA

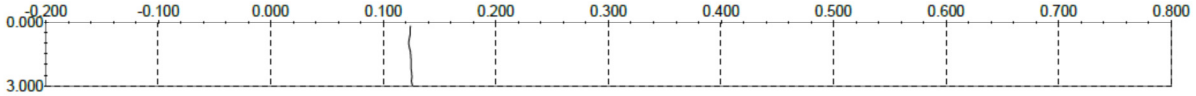
Std-3 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.0000	1.0207	0.0820	1.10	0.0009

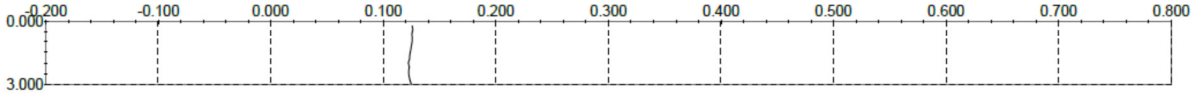
Std-4 : STD



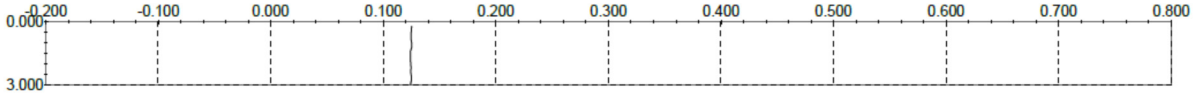
True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.4051	0.1129	05/10/2021	1:15:50 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:30 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5083	0.1212	05/10/2021	1:16:34 PM(+0600)	AA

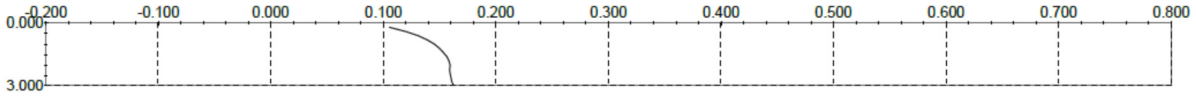


True Value	Conc.	Abs.	Date	Time	Device Name
1.5000	1.5121	0.1215	05/10/2021	1:16:38 PM(+0600)	AA

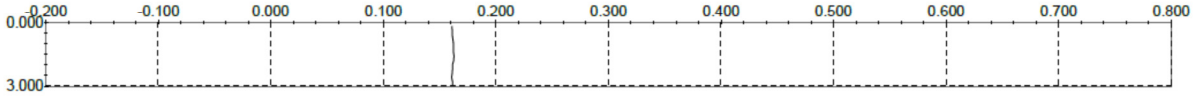
Std-4 : STD Average

True Value	Conc.	Abs.	%RSD	SD
1.5000	1.5096	0.1213	0.14	0.0002

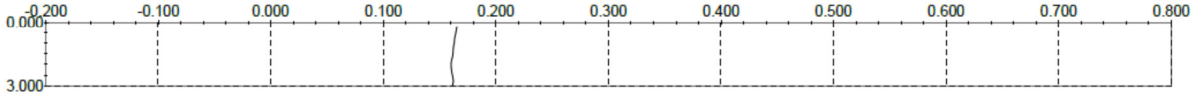
Std-5 : STD



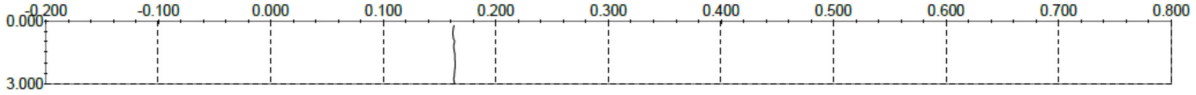
True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.8032	0.1449	05/10/2021	1:16:45 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9736	0.1586	05/10/2021	1:17:25 PM(+0600)	AA



True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9811	0.1592	05/10/2021	1:17:29 PM(+0600)	AA

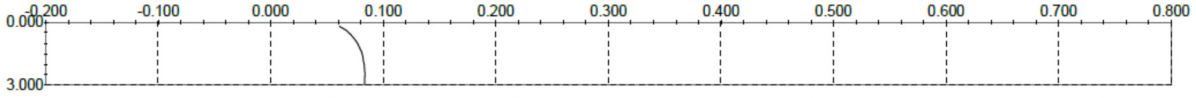


True Value	Conc.	Abs.	Date	Time	Device Name
2.0000	1.9898	0.1599	05/10/2021	1:17:33 PM(+0600)	AA

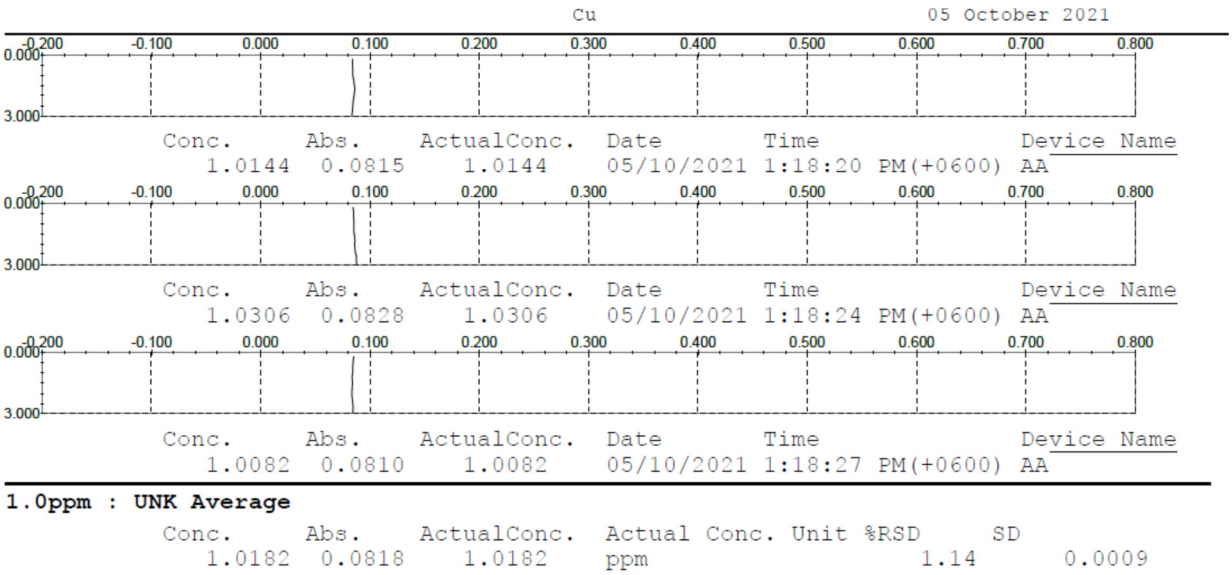
Std-5 : STD Average

True Value	Conc.	Abs.	%RSD	SD
2.0000	1.9811	0.1592	0.41	0.0007

1.0ppm : UNK



	Conc.	Abs.	ActualConc.	Date	Time	Device Name
	0.9361	0.0752	0.9361	05/10/2021	1:17:40 PM(+0600)	AA



Sl. No	Area	Conc(mg/L)	Sample Weight(g)	Conc(mg/kg)
1	0.0410	0.5034	0.5140	9.7934
2	0.0408	0.5009	0.5002	10.0135
3	0.0404	0.4959	0.5011	9.8956
4	0.0411	0.5046	0.5142	9.8139
5	0.0418	0.5134	0.5047	10.1723
6	0.0398	0.4884	0.5022	9.7243
7	0.0401	0.4921	0.5019	9.8050
8	0.0399	0.4896	0.5177	9.4573
9	0.0404	0.4959	0.5101	9.7210
10	0.0404	0.4959	0.5014	9.8897
			STDEV(s)	0.1894
			Average	9.8286
			Precision limit	**0.5303**
			RSD%	**0.1927**

Discussion and Conclusion

In this newly developed improved method for analysis of Copper in bakery products, Atomic Absorption Spectrophotometer (AAS) was used. The Biscuit was used as representative matrix for method validation. International guide line Eurachem was used as method validation protocol. All method validation performance characteristics: Selectivity, Limit of Detection (LOD), Limit of Quantification (LOQ), Working Range and Linearity, Accuracy (Recovery) and Precision (Repeatability) were fulfilled for this matrix. The method is sufficiently accurate, precise, robust and safe for use in BSTI laboratory. Now the method is ready for routine analysis.

References:

1. The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, Second Edition 2014

2. AOAC Official Method 999.10-Copper in Foods, Atomic Absorption Spectrophotometry after Microwave Digestion