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Research Completion Report

Research Proposal:

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

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Research Proposal

Sl. No. & Title	Description
1. Title of the	Development of an improved method in BSTI for analysis of Copper in Bakery products by
Research	Atomic Absorption Spectrophotometer(AAS)
2. Research	In recent years, copper (Cu) enters in agricultural soils, due to arbitrary use of pesticides,
Problem	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	Presently in BSTI Copper is not determined in Bakery product because it is not included in
Previous	Bangladesh Standard Specification but there might be a chance of having Copper in Bakery
Research	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 " <i>fit for purpose</i> ". 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

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

x' = is the mean value of the spiked sample, \overline{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.



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

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



Cu

Std-	4 : 5	TD									
0.000		-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
3.000		i		L	i						
True	Valu	ie (Conc.	Abs.			Date	Time		Device	Name
1	.5000)	1.4051	0.1129			05/10/2021	1:15:50	PM(+0600)	AA	
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1	.5000)	1.5083	0.1212			05/10/2021	1:16:30	PM(+0600)	AA	
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1	.5000)	1.5096	0.1213					0.14	0.0	002
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Sta-:	5:5	STD	0 000	0.400	0.200	0.20	0.400	0.500	0.000	0 700	0.000
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True	Valu	le	Conc.	Abs.			Date	Time		Device	Name
2	.0000)	1.8032	0.1449			05/10/2021	1:16:45	PM(+0600)	AA	
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3 000				L					j		
True	Valu	ie (Conc.	Abs.			Date	Time		Device	Name
2	.0000)	1.9736	0.1586			05/10/2021	1:17:25	PM(+0600)	AA	
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True	Valu	le	Conc.	Abs.			Date	Time		Device	Name
2	.0000)	1.9898	0.1599			05/10/2021	1:17:33	PM(+0600)	AA	
Std-	5 : S	TD	Average								
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2	.0000)	1.9811	0.1592					0.41	0.0	007
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0.000 [±]		-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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3 000											
5.000		(Conc.	Abs.	ActualCor	nc.	Date	Time		Device	Name
			0.9361	0.0752	0.9361		05/10/2021	1:17:40	PM(+0600)	AA	



We used the below formula for calculation of LOD and LOQ



 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



sta-	1 : STD	Average								
True	Value	Conc.	Abs.					%RSD	SD	
0	.0000	-0.0169	-0.0014					4.22	0.	0001
Std-	2 : STD									
0,000	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
0.000			\backslash							
2 000										
True	Value (Conc.	Abs.		Dat	e	Time		Devic	e Name
0	.5000	0.4521	0.0363		05/	10/2021	1:14:01	PM(+0600) AA	
0 000	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
1										
3 000										
True	Value (Conc.	Abs.		Dat	e	Time		Devic	e Name
0	.5000	0.5044	0.0405		05/	10/2021	1:14:41	PM(+0600) AA	
0.000	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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True	Value	Conc.	Abs.		Dat	e	Time		Devic	e Name
0	.5000	0.4994	0.0401		05/	10/2021	1:14:45	PM(+0600) AA	
0.000	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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True	Value	Conc.	Abs.		Dat	e	Time		Devic	e 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									
-0,200	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
0.000	-0.10	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
0.000	-0.10	0 0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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rue Va	alue Cor	nc.	Abs.		Dat	e	Time		Devi	ce Name
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rue Va	alue Cor	nc.	Abs.		Dat	e	Time		Devi	ce Name
1.50	000	1.5083	0.1212		05/	10/2021	1:16:30	PM(+0600)	AA	
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1.50	000	1.5083	0.1212		05/	10/2021	1:16:34	PM(+0600)	AA	
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rue Va	alue Cor	nc.	Abs		Dat		Time		Devi	e Name
1.50	000	1.5121	0.1215		05/	10/2021	1:16:38	PM(+0600)	AA	Jo Hamo
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'rue Va	alue Cor	nc.	Abs.		Dat	e	Time		Devi	ce Name
2.00	000	1.8032	0.1449		05/	10/2021	1:16:45	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
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'rue Va	alue Cor	nc.	Abs.		Dat	e	Time		Devi	ce Name
2.00	000	1.9736	0.1586		05/	10/2021	1:17:25	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
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rue Va	alue Cor		Abs		Dat	. <u>Ф</u>	Time		Devi	e Name
2.00	000	1.9811	0.1592		05/	10/2021	1:17:29	PM(+0600)	AA	Jo Hamo
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rue V	alue Cor		Abs		Det	· •	Time		Devi	
2.00	$\frac{1}{000}$	1.9898	0.1599		05/	10/2021	1:17:33	PM(+0600)	AA	le name
		2.0000	0.1000		00/	20/2021				
ta-5	: STD A	verage	7.1				1			
rue Va	alue Cor	10.	Abs.					*KSD 41	5D	0007
2.00	000	1.9811	0.1592					0.41	0.	.0007

Trueness (Spike Recovery)



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 : ST	D									
-0,200	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
ł				\backslash							
2 000											
True	Value	Co	nc.	Abs.		Dat	e	Time		Device	Name
0	.5000	00	0.4521	0.0363		05/	10/2021	1:14:01	PM(+0600) AA ((Itomo
	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
0.000				1							-
ŧ											
3.000]
True	Value	Co	nc.	Abs.		Dat	:e (1.0.(0.0.0.1	Time	DMALOCON	Device	Name
0	.5000		0.5044	0.0405	0.000	05/	10/2021	1:14:41	PM (+0600	J) AA	0.000
0.000	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
ŧ											
3.000									i		
True	Value	Co	nc.	Abs.		Dat	e	Time		Device	Name
0	.5000		0.4994	0.0401		05/	10/2021	1:14:45	PM(+060)	AA (C	
0 000	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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3.000	Volue	Cr	n a	Aba		' D +				Dorrige	Nome
True	5000	CO	0 5143	ADS.		<u>Dat</u>	.e (10/2021	1.14.40	DM (+060)	Device	Name
0	.3000		0.5145	0.0415		007	10/2021	1.14.49	PH(10000)) AA	
Std-	2 : ST	DA	verage								
True	Value	Co	nc.	Abs.					%RSD	SD	
0	.5000		0.5056	0.0406					1.50	0.0	006
		_									
Std-	3 : ST	D	0.000	0.400			0.400	0.500	0.000	0.700	0.000
0.000	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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3.000		L	l	<u>\</u>					i		
True	Value	Со	nc.	Abs.		Dat	e	Time		Device	Name
1	.0000		0.7221	0.0580		05/	10/2021	1:14:56	PM(+060)	AA (C	
0 000+	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
3.000	Value	Co	n.a.	Aba		 Dot				Dorrigo	Nomo
11ue	0000	00	1 0310	0 0020		Dat 05.	.e (10/2021	1.15.34	DM (+060)	DeATCe	Name
_0 200	.0000.	100	1.0510	0.0829	0 200	0.300	0.400	0.500	0.600	0 700	0.800
0.000			0.000		0.200	0.300	0.400	0.300	0.000	0.700	
ŧ											
3.000 [±]											
True	Value	Со	nc.	Abs.		Dat	e	Time		Device	Name
1	.0000		1.0095	0.0811		05/	10/2021	1:15:40	PM(+060)	AA (C	
0.000	-0.	100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
Ŧ											
3 000											
True	Value	Co	nc	Abs		Det	.0	Time		Device	Name
1	.0000	00	1.0194	0.0819		0.57	10/2021	1:15:43	PM(+0600)) <u>AA</u>	nume
1			1.0101	0.0010		007	10/2021	1.10.10		- /	
Std-	3 : ST	DA	verage								
True	Value	Co	nc.	Abs.					%RSD	SD	
1	.0000		1.0207	0.0820					1.10	0.0	009

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Std-4	4 : S	TD									
0.000		0.100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
3.000		<u>_</u>		i	i			j	j		
True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
1.	.5000		1.4051	0.1129			05/10/2021	1:15:50	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		1					• • • • • • • •				•••
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3.000 ^L			l		I			I			
True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
1.	.5000	_	1.5083	0.1212			05/10/2021	1:16:30	PM(+0600)	AA	
0.000		0.100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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3.000		j	i	i \	i	i	į	i	i		
True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
1.	.5000		1.5083	0.1212			05/10/2021	1:16:34	PM(+0600)	AA	
0.000200		0.100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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True	vaiu 5000	<u>e</u> (1 5101	ADS.			Date 05/10/2021	1.16.20	PM (+0600)	Devi	ce Name
	. 5000		1.0121	0.1215			03/10/2021	1:10:50	FII(+0000)	AA	
Std-4	4 : S	TD	Average								
True	Valu	e C	Conc.	Abs.					%RSD <u>s</u>	SD	
1.	.5000		1.5096	0.1213					0.14	0	.0002
Std-5	5 : S	TD	0.000	0.400	0.000	0.000	0.400	0.500	0.000	0.700	0.000
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3.000		ĺ.		į	i			İ	İ		
True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
2.	.0000		1.8032	0.1449			05/10/2021	1:16:45	PM(+0600)	AA	
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True	Valu	0 (onc	Abs			Dato	Time		Devi	ce Name
11ue 2	0000	- 0	1 9736	0 1586			05/10/2021	1.17.25	PM(+0600)		ce Name
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True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
2.	.0000		1.9811	0.1592			05/10/2021	1:17:29	PM(+0600)	AA	
0.000t		0.100	0.000	0.100	0.200	0.300	0.400	0.500	0.600	0.700	0.800
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True	Valu	e C	Conc.	Abs.			Date	Time		Devi	ce Name
2.	.0000		1.9898	0.1599			05/10/2021	1:17:33	PM(+0600)	AA	
Std-	5 : 5	TD	Average								
True	Valu		'onc	Aba					& D G D G	3D	
2.	.0000	<u> </u>	1.9811	0.1592					0.41	0	.0007
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			conc.	Abs.	ActualCon	nc.	Date	1.17.40	DM (10 C00)	Devi	ce Name
			0.9361	0.0752	0.936.	L	00/10/2021	/ : 40	FM(+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