RESEARCH ARTICLE

Estimation of Added Urea in the Food Products and Marketed Formulation using 1,2-Napthoquinone-4-Sulphonate Reagent by UV- Visible Spectrophotometer

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ABSTRACT
Milk naturally contains urea, which makes up a large portion of the amount of non-protein nitrogen found in the milk. The amount of urea in milk varies throughout herd members. Following that, urea and the Folin (NQS) solution mix to form a vibrant complex that is visible as an absorbed range of 454 nm.
Following optimization, the limit of detection and quantification limitation were found to be the optimal experimental variables, at 0.00280 and 0.00848 µg/ml, respectively. The calibration plot for urea shows a straight line in a concentration that ranges from 0.1–10 µg/mL. The coefficient of the determination was R2 = 0.9999.
Keywords: Urea, 1,2-napthoquinone-4-sulphonate, UV Spectrophotometer.
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INTRODUCTION

Urea

The most common types of adulterants identified in milk include water, fat that has been removed, powdered skim milk added, reconstituted milk, and thickeners such urea and glucose. When the urea content in milk exceeds 70 mg/100 ml, it is referred to as "added urea" and is considered adulterated milk. The extra urea degrades milk quality and causes serious adverse effects such acidity, diarrhoea, kidney failure, intestinal tract damage, and digestive system damage.[1-4]

Mechanism of Action

As the primary amine group reacts with 1,2 – Napthoquinone – 4 – sodium salt in the presence of sodium hydroxide and heated to 70°C. Where sulfone group is replaced with amine group and forms the reddish – orange coloured complex.

MATERIALS AND METHODS

Chemicals Required

Urea, 0.1% 1,2 – Napthoquinone-4-sulphonate sodium salt(Folin's) reagent**,** 0.01M Sodium hydroxide,Buffer solution of pH12 and Distilled Water.

Instruments Required

Weighing balance, pH meter and double beam UV-Visible Spectrophotometer.[5-7]

Preparation of Reagents

Buffer Solution of pH12

Weighed 0.04 gm of Sodium Hydroxide in 100ml Volumetric flask and makeup with distilled water.

0.1% NQS Reagent

A fresh 50ml volumetric flask was filled with 0.05g of NQS reagent, and the remaining volume was filled with distilled water.

Preparation of Standard Stock Solution of Urea

Taken 0.05g of Urea pure drug in 50ml volumetric flask, dissolve and make up with distilled water. The following dilutions are made further.[8]

EXPERIMENTAL METHODOLOGY

Pour 2 millilitres of the pH buffer solution (pH 12) into

Table 1: Quantification

S.No	Milk products	Sample Absorbance	Concentration
1	M1	0.5625	38.9 µg/ml
2	M2	0.6410	45.0 µg/ml
3	M3	0.4384	29.3 µg/ml
4	M4	0.4421	29.5 µg/ml
5	M5	0.3944	25.8 µg/ml
6	M6	0.8136	58.9 µg/ml
7	M7	0.7235	51.4 µg/ml
8	M8	1.8910	141.9 µg/ml
9	M9	0.2412	14.0 µg/ml
10	M10	1.3799	102.2 µg/ml
11	M11	1.6141	120.44 µg/ml
12	M12	3.2377	246.30 µg/ml
13	M13	0.7455	53.1 µg/ml
14	M14	1.6196	120.8 µg/ml
15	M15	1.9402	145.7 µg/ml

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Table 2: Linearity of Urea

Concentration	Absorbance
10ppm	0.1799
25ppm	0.3817
30ppm	0.4494
50ppm	0.7042
70ppm	0.9689
75ppm	1.0267
90ppm	1.2187
110ppm	1.4687
130ppm	1.7276

Table 3: Precision Method of Urea

No. of Repetition	Concentration	Absorbance
1.	50 ppm	0.7025
2.	50 ppm	0.7028
3.	50 ppm	0.7039
4.	50 ppm	0.7082
5.	50 ppm	0.7096
6.	50 ppm	0.7027
Average	Mean	0.7047
Standard Deviation	SD	0.00001095
%RSD	%RSD	0.0015

Table 4: Precision with respect to Intra day

S.No	Concentration(ppm)	Absorbance(nm)	
		Analyst	Analyst
1.	50 ppm	0.7215	0.6923
2.	50 ppm	0.7237	0.6945
3.	50 ppm	0.7218	0.6928
4.	50 ppm	0.7238	0.6965
5.	50 ppm	0.7259	0.6989
6.	50 ppm	0.7282	0.6935
7.	Mean	0.7239	0.6947
8.	SD	0.00000682	0.000006346
9.	%RSD	0.000942	0.000009134

Table 5: Precision with Respect to inter Day

Concentration (µg/ml)	Absorbance Day - 1	Absorbance Day - 2
50	0.7025	0.7012
50	0.7068	0.7056
50	0.7032	0.7028
50	0.7045	0.7039
50	0.7089	0.7078
50	0.7028	0.7014
Mean	0.7047	0.7037
SD	0.000006558	0.0000065
%RSD	0.00093	0.00092

0.1 millilitres of the standard solution (urea). After that, add 2 millilitres of NQS reagent, and let it warm up. For an hour, 70°C is the ideal temperature, and orange-red

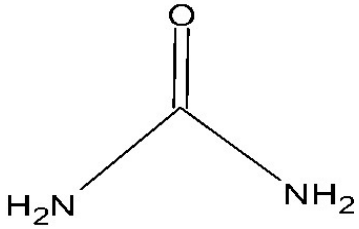


Figure 1: Urea

colour is what is seen. The absorption coefficient is 0.9604 and its wavelength is 454 nm.[9-11]After the many trials, for the optimized trial the wavelength was found to be 454 nm and the absorbance is 0.9604. The optimized pH was pH12 colour was stabilized for 2hours, therefore the colour was optimized and the colour was found to be reddish orange colour.

Method Optimization with pH Value

Colour Stability with Respect to Time

I have checked for every half-hour with against reagent blank, the absorbance was stable for 2 hours.

Extraction Method

Fill a fresh test tube with 5 millilitres of milk. Measure out and add two millilitres of the Folin reagent to your test tube after adding two millilitres of the buffer solution. Additionally, it is heated for 20 minutes at 70°C. The absorbance of the extract was measured at 449 nm.[12-15]

Marketed Formulation of Milk Products

Sample Solution Preparation

Remove one capsule's shell, then weigh the amount of powder inside. 0.005g of powder was weighed, followed by diluting with distilled water in a clean 10ml volumetric flask. 1 ml should be pipetted out into a brand-new 10 ml volumetric flask, followed by 2 ml of the buffer mixture and 2 ml of Folin reagent. Additionally, it is heated for 15 minutes at 70°C. At 454 nm, the absorbance was computed.[16-19]

Y= MX+C

X= 50.63 µg/mL

Recovery % =50.63 * 100/ 50=101.26%

RESULTS AND DISCUSSION

Validation Parameters for Method Guidelines

This technique was verified in accordance with ICH specifications Q2R1. In accordance with the requirements of the International Conference on Harmonization (ICH), the validation parameters were established.[20-23]

RESULTS

The correlation coefficient, or r2, was determined to be 0.9999 and to be within the acceptable range.

Precision

Results of the preparation of six times 6 solutions are listed below. It was discovered that the Urea %RSD of repeatability was 0.0015. RSD for intra-day precision was determined to be 0.3127 in the morning and 0.152279 in the evening. Similarly, the percentage RSD for inter-day precision was determined to be 0.00093 on day 1 and 0.00092on day 2. All of those results, as per ICH criteria, were within the acceptable range.

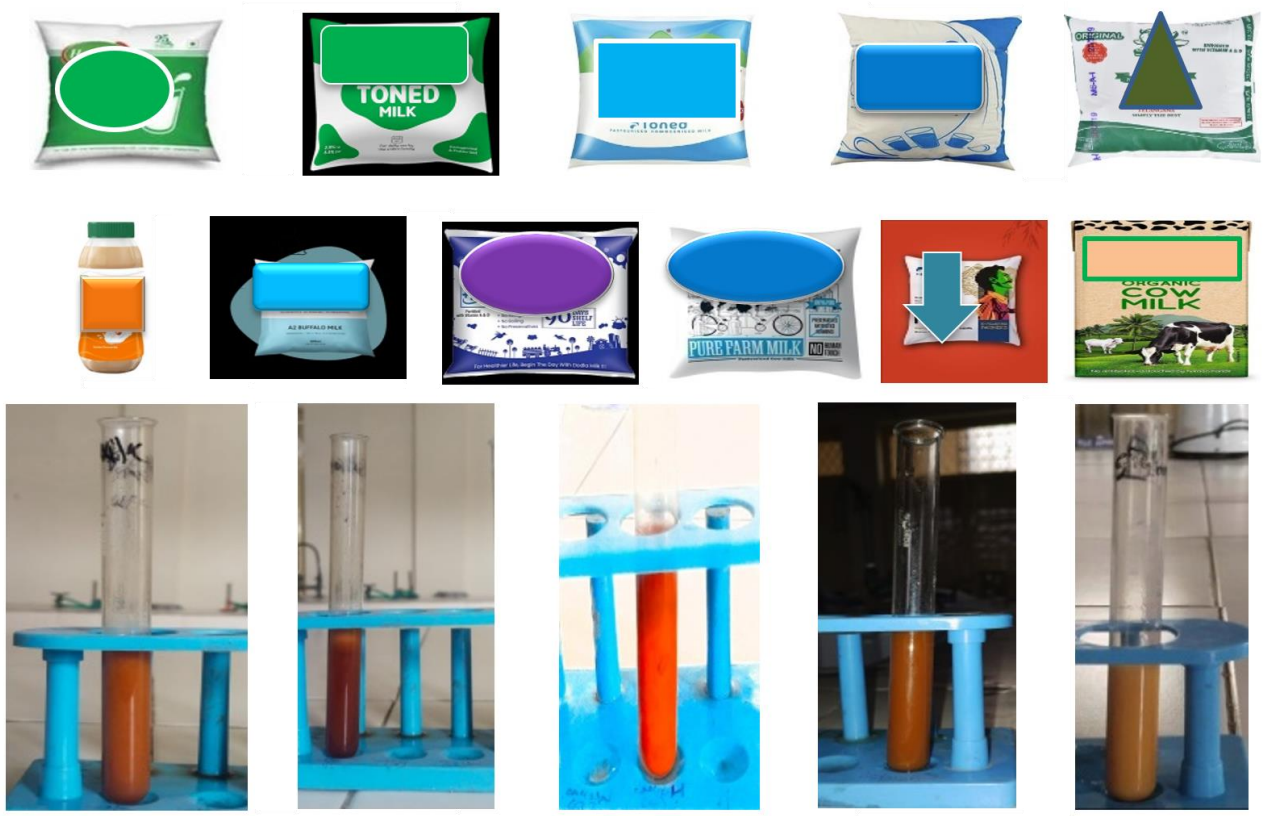
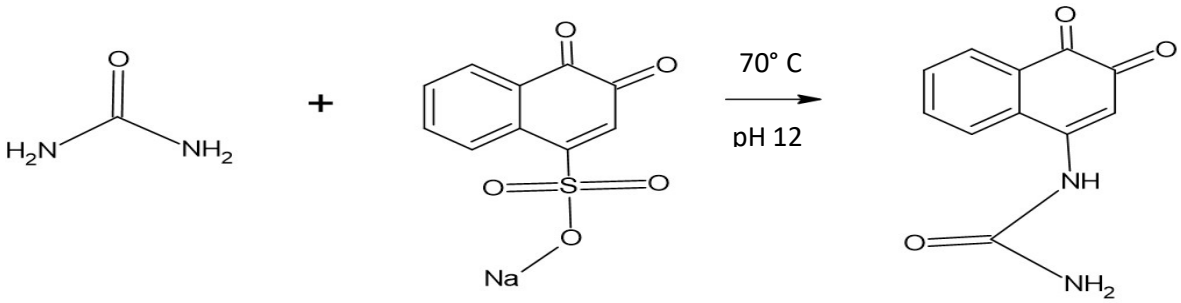


Figure 2: Extraction method and source



Urea | 1,2 – Napthoquinone-4- sulphonate Sodium Salt | Reddish –orange Coloured Complex

Figure 3: Chemical reaction

Accuracy

50%: 25 ppm (standard) spiked to 50 ppm (sample); 1 ml of the spiked standard solution and 1 ml of the sample were pipetted into a 10-ml volumetric flask.

100%: 50ppm (standard) spiked to 50ppm (sample); 1 ml of the spiked standard solution and 1 ml of the sample were pipetted into a 10-ml volumetric flask.

150%: 75ppm (standard) spiked to 50 ppm (sample); 1 ml of the spiked standard solution and 1 ml of the sample were pipetted into a 10-ml volumetric flask. Three degrees of additions of chemicals and reagents (50%, 100%, and 150%) were made. 1ml NQS reagent, 1ml of pH12 buffer. Make up the volume up to the mark with water and colour appears to be reddish orange coloured.

LIMITS

%Recovery should be 98-102% as per ICH guidelines

The Results

The accuracy recovery range was determined to be 99.7–100%, which is within the ICH (98–102%) criteria.

Robustness and Ruggedness

Two separate analysts conducted the ruggedness analysis; the results were Analyst1%RSD0.0011 and Analyst2%RSD 0.001. We examined the robustness using two wavelengths, +1(%RSD 0.000503) along with -1(%RSD 0.000537), and the findings were within the acceptable ranges according to ICH guidance.

The limits of Quantification (LOQ) and Limits of Detection (LOD)

It was determined that the limits of detection (LOD) and limits of quantification (LOQ) were, respectively, 0.00280µg/ml and 0.00848µg/ml.[24-30]

Table 6: Recovery Study of Urea

Percentage Level	Sample Absorbance	Spiking Absorbance	Total Absorbance	% Recovery	Mean % Recovery
50 % (50ppm+25ppm)	0.7042	1.0859	1.0828	98.52%	98.12%
			1.0818	98.16%	
			1.0808	98.02%	
100% (50ppm+50ppm)	0.7042	1.4084	1.4056	99.78%	99.56%
			1.4039	99.32%	
			1.4018	98.53%	
150% (50ppm+75ppm)	0.7042	1.7309	1.7306	99.56%	100.0%
			1.7304	99.31%	
			1.7301	98.24%	

Table 7: Robustness studies at 1nm

Concentration (µg/ml)	Absorbance at 453nm	Absorbance at 455nm
50 ppm	0.8623	0.9732
50 ppm	0.8642	0.9765
50 ppm	0.8667	0.9778
50 ppm	0.8683	0.9792
50 ppm	0.8639	0.9756
50 ppm	0.8657	0.9743
Mean	0.8651	0.9761
SD	0.00000465	0.000004912
%RSD	0.000537	0.000503

Table 8: Ruggedness studies with different analyst and instrument were studied

Concentration (µg/ml)	Analyst–1 Absorbance	Analyst–2 Absorbance
50	0.6923	0.7127
50	0.6938	0.7142
50	0.6952	0.7162
50	0.6968	0.7173
50	0.6987	0.7188
50	0.6994	0.7197
Mean	0.6960	0.7164
SD	0.000007732	0.000007206
%RSD	0.0011	0.001

Table 9: Limit of Detection and Limit of Quantification of Urea

Drug name	LOD	LOQ
Urea	0.00280µg/ml	0.00848µg/ml

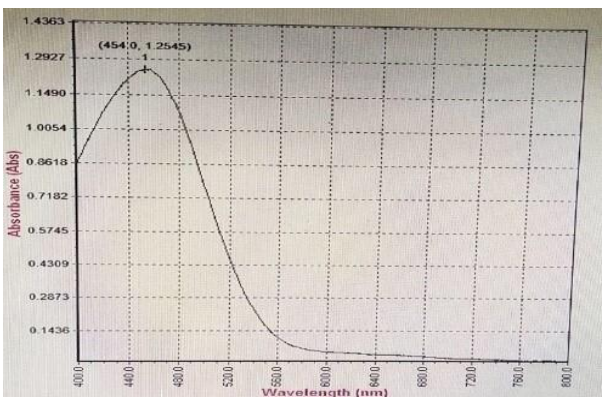


Figure 4: Wavelength

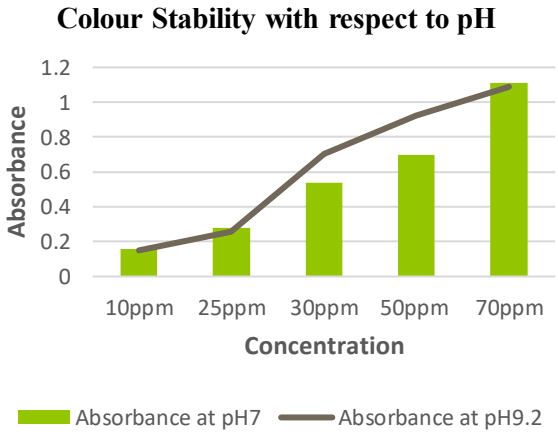


Figure 5: Method optimization with pH Values

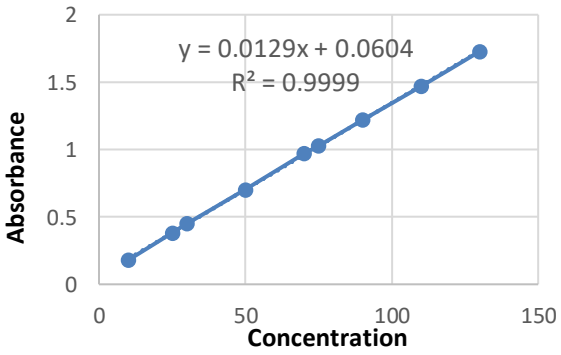


Figure 6: Linearity graph of urea

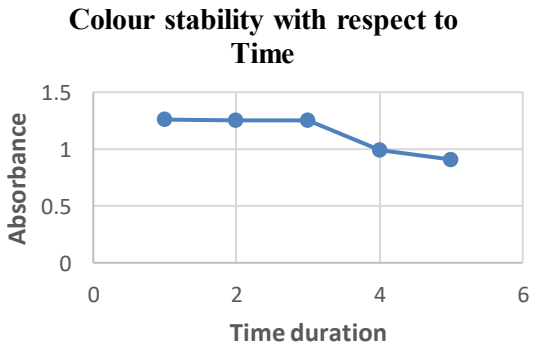


Figure 7: Colour Stability with respect to Time

CONCLUSION

This study set out to create and test a straight forward technique for measuring Urea in a variety of milk products. Analytical validation was made simple, cost-effective, and precise with the above-mentioned method. The developed method is helpful for routine analysis because the results are within the ranges.

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