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DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF OXALIC ACID

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ABSTRACT

New, simple, sensitive, accurate, and specific spectrophotometric method has been developed for the determination of oxalic acid. The developed method, is a chemical derivatization method involving the action of a free carboxylic acid in oxalic acid, sodium nitrite releases nitrous acid which diazotizes sulfanilamide, and a red diazonium salt is developed with α- aphthylamine. exhibiting maximum absorption (λ max) at 482 nm. The effects of reaction time, reagent concentration and volume on the absorption of the complexes formed have been examined. Beer's law is obeyed over concentration ranges of (2-48) µg/ml (R2= 9972). The precision as RSD > 2%, and accuracy was determined the value of the recovery (99.66)%. The equivalent acid content was measured volumetrically by potassium permanganate for comparison and to ensure the accuracy and validity of the method.

KEYWORDS: oxalic acid, chemical derivatization, spectrophotometr, α-naphthylamine.

INTRODUCTION

Oxalic acid is a dicarboxylic acid found in all kinds of life including microorganisms, plants, and animals.[1] In plants oxalates play role in ion balance, plant defense, tissue support, detoxification, light gathering and reflection.[2] The main problem in nutritional exploitation of green leafy vegetable is the presence of anti-nutrients such as oxalic acid. Oxalic acid is associated with metabolic disorders and infectious diseases.[3] Dietary intake of a large amount of oxalic acid could be harmful and leads to the oxalosis or formation of calcium oxalate deposits in vital tissues or organs of the body.[4] Oxalic acid is a primary chelator of

calcium. Hence, its effect in the human diet is very important. Because, oxalic acid can complex with calcium to form highly insoluble calcium oxalate crystals.[5]

In plants, oxalic acid and oxalates have been detected in varying quantities in all parts such as leaves, leaf stalks, flowers, tubers and roots.[6] The highest oxalic acid concentration commonly occurs in the leaves and that lowest in the roots.[7] In plants, oxalates may be present as the soluble sodium or potassium salts or as insoluble crystalline calcium oxalate. The formation of oxalate is typically intracellular, with the crystals forming specialized cells called crystal idioblasts. For many years, oxalic acid was considered as a metabolic end product and it was thought that calcium oxalate was formed to maintain low soluble level of this toxic acid.[8] recent studies have shown that oxalic acid is synthesized in response to increased calcium.[9]

There have been many methods for the determination of oxalic acid. Although potassium permanganate titrimetric analysis of oxalic acid is the cheapest way, this chemical analytical method is time-consuming. Thus, various instrumental analytical techniques such as gas chromatography, liquid chromatography[10] flow-injection catalytic spectrophotometry,[11] ion exclusion chromatography[12] and enzymatic methods[13] have been developed to quantify oxalic acid or oxalate species. However, these instrumental methods have suffered from some disadvantages such as high cost, low sensitivity and insufficient selectivity. This work presents the studies on the analysis of oxalic acid using a chemical derivatization method involving the action of a free carboxylic acid in oxalic acid.

MATERIALS

oxalic acid (Pure ≥99% , Sigma-Aldrich. Germany).
acetone (pure ≥ 99.9%, Sigma-Aldrich. Germany).
Sulfanilamide (pure ≥ 99%, Sigma-Aldrich. Germany).
Sodium nitrate (pure ≥ 99%, Sigma-Aldrich. Germany).
alpha-naphtalamine (pure ≥ 99%, Sigma-Aldrich. Germany).

Apparatus

Cecil, Ce7200 double beam UV-Visible recording spectrophotometer with pair of 1 cm matched quartz cell was used to measure absorbance of solutions.
Ultrasonic bath, Branson 200.
A SHIMADZU analytical balance with 0.01 mg.

Standard solution and reagent

A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid in distilled water and diluted to 100 ml with distilled water. Working solution was prepared freshly before use.

A Solution of 2% w/v reagent was freshly prepared by dissolving 2g Sulfanilamide in 100 ml acetone (reagent A).

A Solution of 2% w/v reagent was freshly prepared by dissolving 2g Sodium nitrite in 100 ml distilled water (reagent B).

A Solution of 2% w/v reagent was freshly prepared by dissolving 2g alpha-naphtalamine in 100 ml acetone (reagent C).

Procedure for pure standard solution of oxalic acid

Aliquots of the working standard solution of **oxalic acid** were transferred in a series of 50 ml volumetric flask to give final concentrations of 2-48 µg/ml. 100 µl of reagent A +100 µl of reagent B + 50 µl of reagent C were added to each flask and volume was made up to the mark with distilled water. The solutions were shacked well and allowed to stand for 5 minutes to complete the reaction. The absorbance was measured at 482 nm against blank reagent prepared similarly. The Standard solution control concentration was calculated from the corresponding regression equation of the calibration graph.

RESULTS AND DISCUSSION

Absorption spectra

Oxalic acid shows maximum absorption at 482 nm using distilled water as a solvent AFTER derivatization (Figure 1). Upon the action of a free carboxylic acid in oxalic acid, sodium nitrite releases nitrous acid which diazotizes sulfanilamide, and a red diazonium salt is developed with a-naphthylamine (Figure 2).

The absorption spectra of the red colored products were recorded at 380–780 nm against the corresponding blank solution.

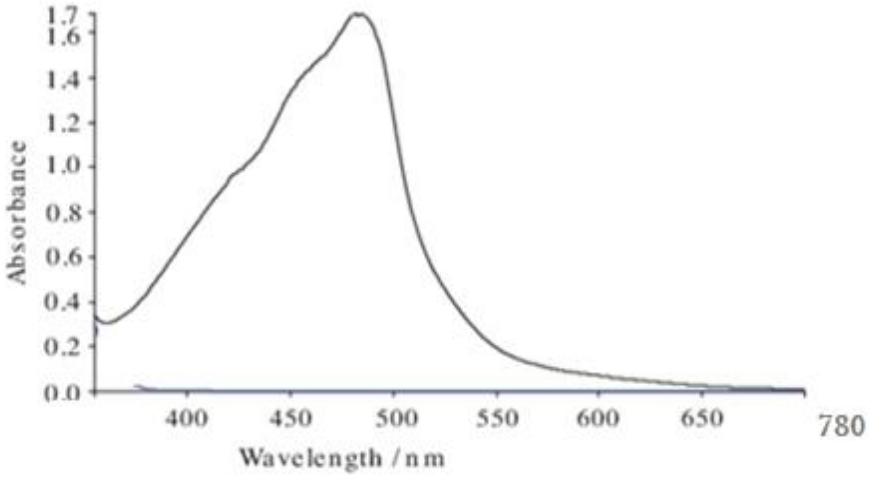

Figure 1: oxalic acid spectra AFTER derivatization.

Stoichiometric relationship

Job's method of continuous[14] variation was employed to determinate the molar ratio between Oxalic acid and alpha-naphtalamine. The concentration of each Oxalic acid and reagent was adjusted to be 2×10^{-3} M. A series of solutions was prepared in which the total volume of the Oxalic acid and the reagent was kept at 2.5ml in 10ml calibrated flasks. The solutions were further manipulated as described under the general recommended procedures described above. The absorbance of each solution (against blank treated similarly) was plotted against the Oxalic acid mole fraction [Oxalic acid]/[Oxalic acid + reagent of alpha-naphtalamine]. The molar ratio was 0.6:0.4 as shown in (Figure 2).

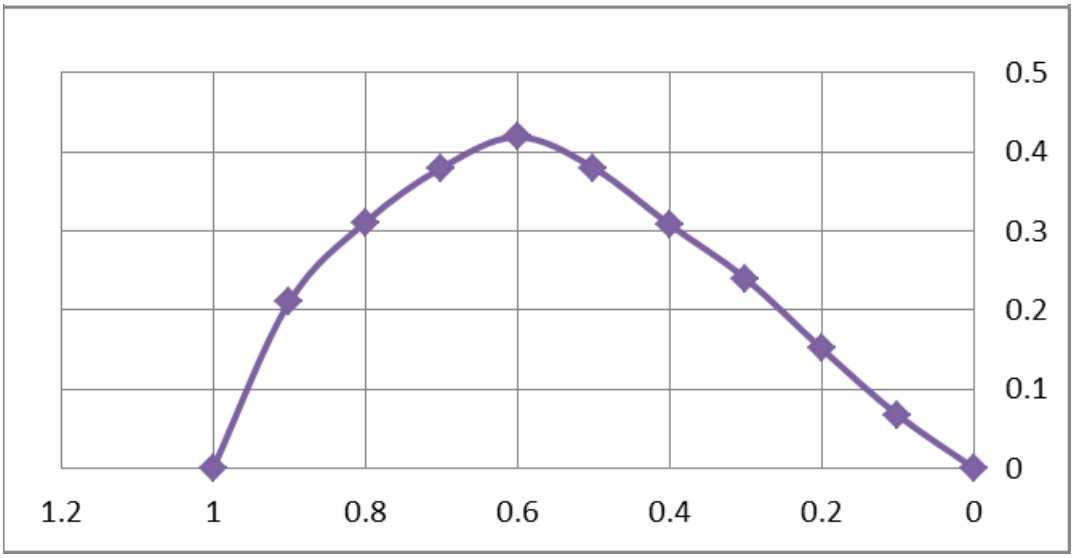

Figure 2: Continuous variation plots of oxalic acid with reagent C.

Validation of the proposed method[15]

Linearity

The correlation coefficient (R^2) of the formed product was 0.9972 indicating good linearity (Figure 3) The limit of detection (LOD) and limit of quantification LOQ) for the proposed method were calculated using the following equations: LOD = 3.3 σ / S, LOQ = 10 σ / S Where σ is the standard deviation of intercept, S is the slope of calibration curve. The results are summarized in table 1.

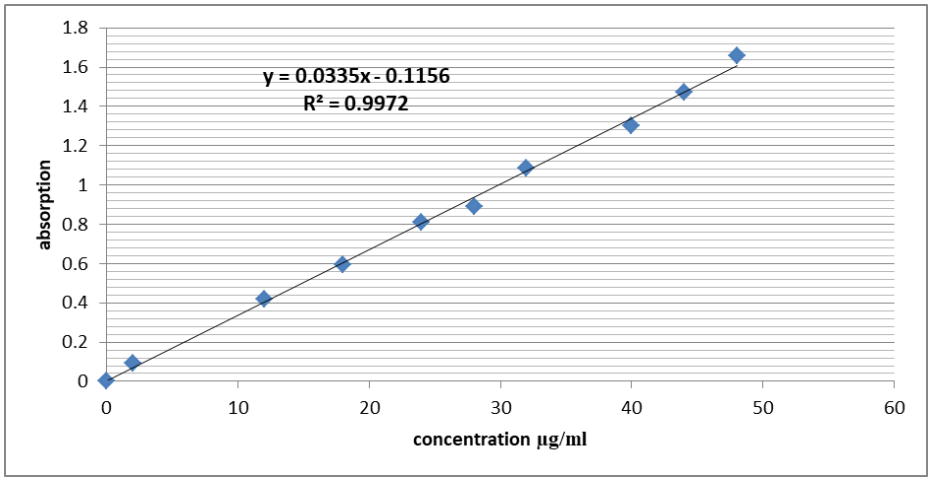

Figure 3: Calibration graph for oxalic acid after derivatization.

Table 1: Quantitative parameters of the proposed method.

Parameter	Value
Time of reaction (min)	5 min
Stability of color (Hour)	1/2 hours
Beer's law limits (µg/mL)	2-48
Regression equation (y=b+ac)*	y = 0.0335x - 0.1156
Correlation coefficient (R2)	0.9972
Slope, a	0.0335
Intercept, b	0.1156
LOD (µg/ml)	0.0501
LOQ (µg/ml)	0.1519

*Y = aX+ b, where X is the concentration of oxalic acid µg ml -1

Table 2: Accuracy and precision of the proposed method for the determination of oxalic acid.

Concentration (µg/ml)	Accuracy* %	Precision			
		Intra- day		Inter –day	
		Recovery ±SD	RSD*	Recovery ±SD	RSD*
12	98.653	99.81±1.932	1.935	99.86±1.94	1.952
18	99.90	95.32±1.97	1.999	95.41±1.70	1.790
24	100.44	96.07±1.82	1.980	98.816±1.92	1.950

*n=5, SD=standard deviation, RSD=Relative standard deviation

Accuracy and Precision

They were checked at three concentration levels, five replicate measurements were recorded at each concentration level. Accuracy was recorded as percent recovery, and by standard addition method. Precision was recorded as relative standard deviation. The calculated relative standard deviations were below 2% indicating good precision of the proposed procedure at both levels of inter-day and intra-day precision; the results are summarized in table 2. The percent of recoveries of OXALIC ACID by standard addition method were in the range of 95.41-99.86 (table 2).

Specificity

The proposed method was compared with the standard method listed in AOAC by potassium permanganate where the results listed in the table were compared. Then the values of F and T were calculated and we got the following values in order :F=1.17, T=0.69

These values are smaller than the tabular values and are also on the order F=6.39, T=2.306 With this we judge that the accuracy is 95%, meaning that the proposed method does not differ in terms of accuracy and reliability with the standard method and that the standard diffraction of both methods is the result of random errors.

Standard method concentration µg/ml	new method concentration µg/ml
23.01	23.33
23.95	24.66
24.21	24.11
23.88	24.25
24.35	24.16

$\bar{X}_1$=23.88 $\bar{X}_2$=24.10

$$s^2_1 = \frac{\sum(Xi1-\bar{X1})^2}{n1-1} = \frac{0.9326}{4} = 0.23$$

$$s^2_2 = \frac{\sum(Xi2-\bar{X2})^2}{n2-1} = \frac{1.09}{4} = 0.27$$

$$F=\frac{V1}{V2}$$

$$F=\frac{0.27}{0.23V2}=1.17$$

$$\mu = \bar{X} \pm \frac{ts}{\sqrt{n}}$$

$$\pm t = (\bar{X} - \mu)\frac{\sqrt{n}}{s}$$

$$s = \sqrt{\frac{\sum(xi1-x1^{-})^2+(xi2-x2^{-})^2}{n1+n2-2}}$$

$$= \sqrt{\frac{0.93+1.09}{5+5-2}} = \sqrt{\frac{2.02}{8}} = 0.50$$

$$\pm t = \frac{0.22}{0.50}\sqrt{\frac{5*5}{5+5}} = 0.69$$

CONCLUSION

A new method which is selective, sensitive, and rapid has been developed and appropriately validated for the assay of oxalic acid. The analytical reagent is inexpensive and readily available in any analytical laboratory. The method does not require complex procedures (extraction step, heating) or sophisticated equipments and is highly suitable for routine use in quality control laboratories.

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