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THE DETERMINATION OF THIAMINE CONTENT IN SEAWEEDS

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Determination of Thiamine in Seaweeds was carried out using improved technique with the help of three different Spectrophotometers. This method was applied on standard sample of thiamine (98.5-101.5%) and seven different species of Seaweeds belonging to Red and Brown family: *Botryocladia microphysa, Carpogonia floridеae, Dictyota dichotoma, Tetrasporangia, Iyengaria stellata, Samia indica* and *Hypnea musciformis*. The method was also compared with other methods such as U.S.P., B.P, Pak. Pharmacopeae.

Key words: Thiamine, Seaweeds, Improved method

Introduction

Thiamine has various medicinal value and is therapeutically used in the treatment of idiopathic anorexia in dogs, for retarded growth of young animals, polyneuritis, convulsions, in disturbance of metabolism and for beri beri disease. William *et. al.* first synthesised thiamine in 1935 [1,2]. For some time after the availability of synthetic thiamine, there was considerable discrepancy as to the extiction co-efficients for this vitamin at different wavelengths. However, it is preferable to measure the thiamine content at wavelength of excitation maximum at 368 nm.

Statistical analysis by spectrophotometeric technique have been studied [3-5]. In this study, the method reported by Fujiwara *et. al.* [6], for the study of thiamine in biological materials was improved to determine the thiamine contents in Seaweeds by spectrophotometeric technique.

Materials and Methods

The seven samples of Seaweeds were collected in the month of December from Monora seaside at Karachi. All the fresh samples were thoroughly washed with fresh water and stored at 4°. Standard of thiamine sample (98.5-101.5% pure) was procured from Hakimsons Chemical Industries (Pvt.) Ltd., Karachi.

Approximately 6 µg of thiamine per ml. is required for spectrophotometeric determination technique, which under ideal circumstances can be measured with probable error of 0.12 µg [7].

The apparatuses used were Spectrophotometers MO, Photic-100, Unicam Pye no. 1046, Beckman 2107. All the samples and standards were read at 368 nm wavelength.

Standard solution. 10% of 0.000104 gm/ml thiamine

Preparation of samples. Fresh sample of Seaweeds were grinded with the addition of small quantity of N/10 HCl in water to make fine residual substance of each species separately, and filtered with filter paper. Slowly and gradually the residue was washed with N/10 HCl till residue becomes colourless and were washed with deionized water. The volume was made upto 100 ml. in a volumetric flask with water. The strength of samples are given in the Table 2.

Chemicals

(a) Freshly prepared Cynogen bromide (CNBr).
(b) 3% Sodium hydroxide.
(c) Isobutanol.
(d) Sodium sulphate.

Method. Four test tubes for each sample/standard were taken and marked as 1,2,3 and blank, (Table 1).

Table 1

Tube no	Standard/ sample	CNBr	30% NaOH	Isobutanol	Na_2SO_4
1.	1 ml	5 ml	5 ml	5 ml	1 gm
2.	1 ml	5 ml.	5 ml	5 ml	1 gm
3.	1 ml	5 ml.	5 ml	5 ml	1 gm

Blank:- 1 ml. of sample + 5 ml. of 30% NaOH + 5 ml. of CNBr + 5 ml. of Isobutanol + 1 gm. Na_2SO_4

Each tube was shaken for about one minute and then placed for 5 minutes. The Isobutanol layer was separated and used for the determination of thiamine at the absorbance of 368nm wavelength. The value of A (1 percentage, 1 cm.) = 151 calculated absorbance shown in Table 2.

Results and Discussion

The improved method was utilized for the determination of thiamine in seven different species of Seaweeds along with standard on three different spectrophotometers at 368 nm wavelength for accuracy as shown in Table 2. The percentage of standard on Beckman is 99.3377, on Unicam 101.8848%, and on MO photic-100 98.7% (thus the average percentage comes to about 99.9741%). Since normally the strength of standard is standardized as 98.5 - 101.5%, the other seven samples of Seaweeds were studied to similar standard method. The calculated value of standard and the percentage of all seven samples of Seaweeds shows the small difference due to standard sample which contians ± 1.5% error. The above method compares well with the other three

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TABLE 2

S.No.	Name of species/ standard	Weight of sample in gm.	Strength of sample gm/ml	Absorbance on Beck man	(%)	Absorbance on unicam	(%)	Absorbance on MO, Photic-100	(%)	Average (%)
1.	Standard	0.104	0.0000104	0.156	99.3377	0.16	101.8848	0.155	98.7	99.9741
2.	*Tetra sporangia*	3.5271	0.035271	0.013	0.00246	0.014	0.00258	0.013	0.002473	0.002504
3.	*Botryocladia microphysa*	1.0314	0.010314	0.002	0.001292	0.002	0.00126	0.002	0.001301	0.001284
4.	*Carpogonia florideae*	1.5725	0.015725	0.025	0.010598	0.026	0.010746	0.025	0.010667	0.01067
5.	*Dictyota dichotoma*	3.1449	0.031449	0.02	0.004329	0.021	0.00434	0.02	0.004267	0.004312
6.	*Iyengaria stellata*	5.0323	0.050323	0.105	0.01391	0.109	0.014078	0.105	0.013999	0.013995
7.	*Samia indica*	7.5454	0.075454	0.013	0.001148	0.014	0.0012	0.013	0.001156	0.001168
8.	*Hypnea muscitormis*	1.1322	0.011322	0.015	0.008832	0.016	0.009185	0.015	0.008889	0.008968

The table shows the readings on three different spectrophotometers of seven species with standard solution. All the readings were taken at 368nm. Table also shows the percentage, average percentage and strength of standard and strength of samples of seven seaweed species.

TABLE 3

Name of Species/ standards	Standard	*Tetra sporangia*	Botyro- *cladia microphysa*	*Carpogonia florideae*	*Dictoyota dichotoma*	*Iyengaria stellata*	*Samia indica*	*Hypnea musci- formis*
Wt. of sample in gms.	0.104	3.5271	1.0314	1.5725	3.1449	5.0323	7.5454	1.1322
Strength of sample gm/ml.	0.0000104	0.035271	0.010314	0.01572	0.031449	0.050323	0.075454	0.011322
Avg.% by new method	99.9741	0.002504	0.001284	0.01067	0.004317	0.013995	0.001168	0.008968
Avg.% by B.P. method	100.2143	0.00251	0.00117	0.01069	0.00433	0.01403	0.00118	0.00899
Avg.% by USP method	98.7245	0.002472	0.0011	0.01053	0.00427	0.01382	0.001163	0.008855
Avg.% by Pak Pharmacopeae method	99.5168	0.00249	0.00116	0.01061	0.00429	0.01393	0.00117	0.00893
Mean% of 3 Reported methods	99.4855	0.00249	0.00114	0.01061	0.00429	0.01393	0.00117	0.00892

Note: Absorbance were recorded on different spectrophotometers (Table 2) for checking the possible errors and taking the mean average. As can be observed the difference between standards mean value and blanks mean value, where a difference of 0.4886 can be calculated (99.9741-99.4855).

standard methods such as U.S.P., B.P. and Pakistan Pharmacopeae as shown in Table 3.

It is also evident that the above work for the determination of Thiamine in Seaweeds using Cynogen bromide by improved technique is being reported for the first time.

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