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HPLC and Chemical Methods for the Determination of Vitamin B-Complex in Rice and Rice Products

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Evaluation of high performance liquid chromatography (HPLC) and chemical methods for vitamin B-complex, namely, thiamine, riboflavin and niacin in rice and rice products was carried out. No significant difference in the accuracy of results was observed between two methods of determination for thiamine and riboflavin. But there is significant difference in case of niacin. The study undertaken may find application for the correction and standardization of analysis method for B-vitamins.

INTRODUCTION

Rice is one of the most preferred food grains, which is eaten by large populations worldwide[1]. A number of preparations made from rice are available in the market. Nutritional labelling of food products is gradually becoming mandatory in even developing countries by health and food law regulatory bodies. Thus an accurate and rapid method is required so that in a short time a profile of the status of vitamins present in a product may be obtained for research, standardization and quality assurance purposes.

Several comparative studies of instrumental analysis and wet chemical analysis methods recommended by Association fo Official Analytical Chemists (AOAC) have been carried out for water-soluble vitamins by various scientists[2, 3]. Their findings warrant more comprehensive work on thiamine, riboflavin and particularly on niacin. Methods provided by AOAC for water soluble vitamins are lengthy[4], time-consuming and sometimes require hazardous toxic chemicals, *e.g.*, cyanogen bromide is used for niacin determination.

In view of this, the present study was undertaken to compare the AOAC methods with High Performance Liquid Chromatography for the determination of thiamine, riboflavin and niacin in rice and rice products.

EXPERIMENTAL

Thiamine, riboflavin and niacin were determined by chemical methods as per the methods prescribed by AOAC method.[4]

The HPLC used was Waters Associates Model operated by MAXIMA 820 Workstation equipped with a Waters 501 Pump and Waters 486 U.V. Absorbance Detector. The peaks were obtained by Wipro LX 800 Printer. The chromatography column used was of Waters Associates (3.9 mm × 30 cm) Microbondapack C18. The flow rate was 0.8 mL/min.

Parboiled rice (milled) and raw rice (milled) were taken for the study.

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Simultaneously for determination of vitamins the recipes of both kinds of rice were prepared by boiling the rice in rice-cooker for 12 min in ordinary domestic tap water as in most Asian countries rice is eaten after simply boiling it. The cooked rice was freeze-dried under vacuum for 24 h. Ground samples were passed through 40 mesh sieve and stored in screw-capped moisture-proof containers for analysis.

The HPLC analysis was carried out as suggested by Toma and Tabekhia[5] and Kirk[6] with some modifications. The chemicals used were of HPLC grade.

Spiked samples were obtained by adding known amounts of thiamine, niacin and riboflavin and then they were analysed for their recovery studies. Concentration of each vitamin was measured by UV absorbance at 254 nm.

RESULTS AND DISCUSSION

The elution profiles of B-vitamins are given in Fig. 1. With the help of electronic integration the area of peak was measured and quantitation was carried out. The data so obtained are given in Table-2. From the data, it is evident that in case of thiamine no significant difference was observed and for riboflavin a marginal difference between HPLC and AOAC method was observed.

TABLE-1
PERCENTAGE OF B-VITAMINS RECOVERED FROM ENRICHED SAMPLES

	AOAC Method		HPLC Method	
	Uncooked	Cooked	Uncooked	Cooked
Thiamine	92.07 ± 3.08	92.00 ± 2.74	94.14 ± 4.92	94.28 ± 2.9
Riboflavin	96.12 ± 4.78	95.94 ± 4.29	97.38 ± 5.34	96.5 ± 5.48
Niacin	93.43 ± 5.64	92.70 ± 5.26	96.76 ± 3.18	95.48 ± 4.07

Values are mean of five observations; means ± S.D.

TABLE-2
COMPARISON OF CONTENTS OF B-VITAMINS PRESENT IN RICE AND RICE PRODUCTS BY AOAC AND HPLC METHODS

Vitamin (μg/g)	Raw rice (milled)		Parboiled rice (milled)	
	HPLC method	AOAC method	HPLC method	AOAC method
Thiamine:				
Uncooked rice	1.19 ± 0.05	1.14 ± 0.046	2.29 ± 0.102	2.26 ± 0.104
Cooked rice	0.95 ± 0.02	0.94 ± 0.023	1.86 ± 0.037	1.80 ± 0.043
Niacin:				
Uncooked rice	22.00 ± 0.68	19.00 ± 0.722	42.00 ± 0.785	38.00 ± 1.178
Cooked rice	19.36 ± 0.39	16.53 ± 0.411	36.70 ± 1.350	33.82 ± 1.346
Riboflavin:				
Uncooked rice	0.38 ± 0.027	0.35 ± 0.024	0.47 ± 0.019	0.45 ± 0.037
Cooked rice	0.34 ± 0.014	0.31 ± 0.019	0.41 ± 0.016	0.39 ± 0.036

Values are mean of five observations; mean ± S.D.

But in case of niacin a noticeable difference of 3 μg/g (13.6%) for raw milled

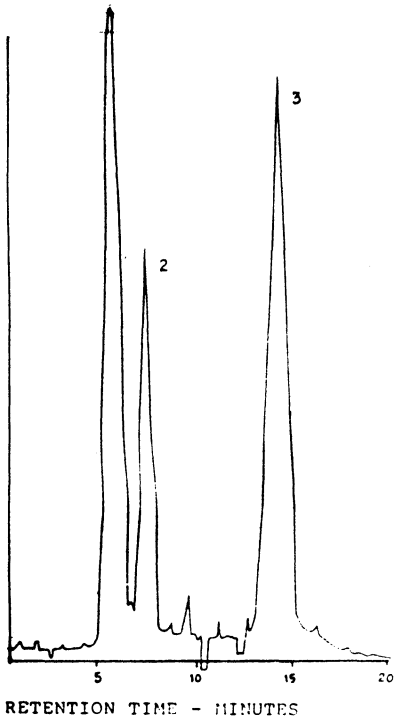

Fig. 1a. Chromatogram of standards of B-vitamins: 1. Niacin; 2. Riboflavin; 3. Thiamine

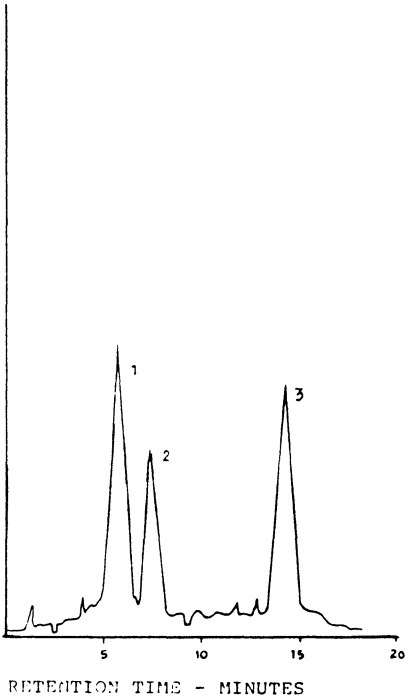

Fig. 1b. Chromatogram of B-vitamins of cooked rice sample: 1. Niacin, 2. Riboflavin; 3. Thiamine

uncooked rice and 4 μg/g (9.5%) for parboiled milled rice was observed in HPLC and AOAC methods respectively. Similarly a difference of 2.83 μg/g (14.6%) for cooked raw milled rice and 2.88 μg/g (7.8%) for cooked parboiled milled rice was observed in HPLC and AOAC methods respectively.

In all cases HPLC method gave higher content of niacin and the difference in result of AOAC method was also significant. The recovery of the vitamins (Table-1) from the enriched rice is in better analytical agreement with HPLC method as compared to AOAC method.

Conclusions

It was experienced that the HPLC method is more convenient with greater accuracy and consumes less time in comparison to chemical analysis method suggested by AOAC. The study also suggests a reassessment of the recommendations of standard method of AOAC for B-Vitamins.

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