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RP HPLC Method for the Estimation of Riboflavin in Various Extracts of Pumpkin

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***Abstract*:-** A simple, rapid, precise and accurate isocratic reversed-phase high performance liquid chromatography (RP-HPLC) method was developed to determine the riboflavin by extrapolation and direct comparison method in pulp, peel and seeds of pumpkin separation was achieved using a 25 x 4.6 mm column, particle size 5 micron C_{18} reverse phase column (Phenomenex), with a mobile phase consisting of methanol, water and 0.01% CH_3COOH, in isocratic elution mode with a mobile phase flow rate of 1 ml/min, using UV visible detection at 270 nm. Regression analysis showed a good linear relationship $r^2 = 0.998$.

***Keywords*-** Riboflavin, Pumpkin, pulp, peel, seed, RP-HPLC, UV.

I. INTRODUCTION

Pumpkin refers to certain cultivars of squash, most commonly those of *Cucurbita pepo*, that are round, with smooth, slightly ribbed skin and deep yellow to orange coloration. The thick shell contains the seeds and pulp.

A pumpkin is a cultivar of a squash plant, most commonly of Cucurbita pepo, that is round, with smooth, slightly ribbed skin, and most often deep yellow to orange in coloration.[1] The thick shell contains the seeds and pulp. Some exceptionally large cultivars of squash with similar appearance have also been derived from Cucurbita maxima. Specific cultivars of winter squash derived from other species, including C. argyrosperma, and C. moschata, are also sometimes called "pumpkin"[1] and is used interchangeably with "squash" and "winter squash".[1]

All pumpkins are winter squash: mature fruit of certain species in the genus Cucurbita. The word pumpkin originates from the word pepon (πέπων), which is Greek for "large melon", something round and large.[2] Characteristics commonly used to define "pumpkin" include smooth and slightly ribbed skin,[3] and deep yellow to orange[3] color. Circa 2005, white pumpkins had become increasingly popular in the United States.[4] Other colors, including dark green (as with some oilseed pumpkins), also exist.

Fig. 1

The French adapted this word to pompon, which the British changed to pumpion and to the later American colonists became known as pumpkin.[5]

The term pumpkin has no agreed upon botanical or scientific meaning,[6] . In North America and the United Kingdom, pumpkin traditionally refers to only certain round, orange varieties of winter squash, predominantly derived from Cucurbita pepo, while in Australian English, pumpkin can refer to winter squash of any appearance.[7]

Pumpkins are a warm-weather crop that is usually planted in early July. The specific conditions necessary for growing pumpkins require that soil temperatures 8 centimetres (3 in) deep are at least 15.5 °C (60 °F) and soil that holds water well. Pumpkin crops may suffer if there is a lack of water or because of cold temperatures (in this case, below 18 °C or 65 °F; frost can be detrimental), and sandy soil with poor water retention or poorly drained soils that become waterlogged after heavy rain. Pumpkins are, however, rather hardy, and even if many leaves and portions of the vine are removed or damaged, the plant can very quickly re-grow secondary vines to replace what was removed.[8]

Pumpkins produce both a male and female flower; honeybees play a significant role in fertilization.[8] Pumpkins have historically been pollinated by the native squash bee Peponapis pruinosa, but this bee has declined, probably at least in part to pesticide (imidacloprid) sensitivity,[9] and today most commercial plantings are pollinated by honeybees. Inadequately pollinated pumpkins usually start growing but abort before full development. Pumpkins are grown all around the world for a variety of reasons ranging from agricultural purposes (such as animal feed) to commercial and ornamental sales,[11]. Pumpkins are, however, rather hardy, and even if many leaves and portions of the vine are removed or damaged, the plant can very quickly re-grow secondary vines to replace what was removed.[12].

Vitamins are essential group of food, which are essential in the diet for maintenance of body cell and normal metabolic functions .They are divided into water-soluble and fat-soluble vitamins. The well-known B-complex belongs to the first group. Vitamin B_2 (riboflavin) has a well-defined process in metabolism of fats, carbohydrates, and respiratory proteins. Riboflavin (also known as vitamin B2) is one of the B vitamins, which are all water soluble. Riboflavin is naturally present in some foods, added to some food products, and available as a dietary supplement. This vitamin is an essential component of two major coenzymes, flavin mononucleotide (FMN; also known as riboflavin-5'-phosphate) and flavin adenine dinucleotide (FAD). These coenzymes play major roles in energy production; cellular function, growth, and development; and metabolism of fats, drugs, and steroids .[13-15]. About 95% of riboflavin in the form of FAD or FMN from food is bioavailable up to a maximum of about 27 mg of riboflavin per meal or dose [15]. The conversion of the amino acid tryptophan to niacin (sometimes referred to as vitamin B3) requires FAD .[15]. The largest dietary contributors of total riboflavin intake in U.S. men and women are milk and milk drinks, bread and bread products, mixed foods whose main ingredient is meat, ready-to-eat cereals, and mixed foods whose main ingredient is grain [15]. Similarly, the conversion of vitamin B6 to the coenzyme pyridoxal 5'-phosphate needs FMN. In addition, riboflavin helps maintain normal levels of homocysteine, an amino acid in the blood. Foods that are particularly rich in riboflavin include eggs, organ meats (kidneys and liver), lean meats, and milk [14, 16]. Green vegetables also contain riboflavin. Grains and cereals are fortified with riboflavin in the United States and many other countries [16] The riboflavin in most foods is in the form of FAD, although the main form in eggs and milk is free riboflavin [17]. The bioavailability of free riboflavin is similar to that of FAD and FMN [17,18]Because riboflavin is soluble in water, about twice as much riboflavin content is lost in cooking water when foods are boiled as when they are prepared in other ways, such as by steaming or microwaving [19]

II. DRUG PROFILE

Vitamin B_2 - Riboflavin

Vitamin B_2 is the name given to riboflavin and related compounds with similar biological activity.

Chemical name : 7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxypentyl)benzopteridine-2,4(3H,10H)-dione

Molecular formula : $C_{17}H_{20}N_4O_6$

Molar mass : 376.364

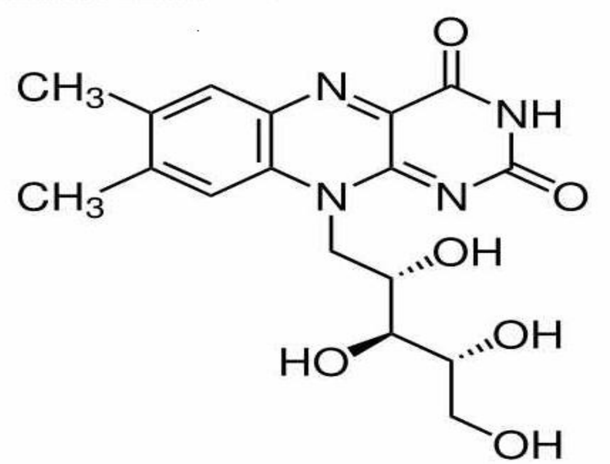

Fig1: Structure of Riboflavin

III. MATERIALS AND METHODS

Sample extraction:
Pumpkin grown was selected, while organically grown vegetable was purchased and used for the extraction of the seeds, pulp and peel from it.

Preparation of samples:
The fresh and healthy vegetables were immediately washed under tap water and excessive water dripped off.
Edible portions (100 g) of the vegetables were cut into small pieces and homogenized using a blender .

Riboflavin:
One gram of sample was weighed and transferred into a 50 ml graduated polypropylene centrifuge tube. Then 17.5 ml of 0.1 N sulphuric acids was added to it. The mixture was shaken vigorously for 1 min, and then placed in boiling water for 30 min and shaken at 10 min intervals. The

mixture was cooled in an ice bath before the addition of 2.5 ml of 2% a-amylase. After a gentle mixing, the mixtures (seeds, pulp & peel) was sonicated for 15 min and kept in water bath for 5 min. The mixture was cooled and then diluted to 25 ml with deionised water. The resulting mixture was spun at 2500 rpm for 15 min at room temperature using a bench top centrifuge.
The supernatant was filtered through a 0.45 µm nylon filter disc before HPLC analysis. All samples were carried out in triplicates.

Materials and Methods
Determination of vitamins by HPLC method
The standards were injected onto an HPLC system using the following conditions:

Column : Genesis C18; 250 x 4.6mm,
Mobile phase : Methanol / water /glacial acetic acid (65/35/0.1)
Flow rate : 1 ml/min

Table 1: Separation conditions

	Pulp	**Peel**	**Seed**
Mobile phases	Methanol-water-acetic acid glacial (65:35:0.1)	Methanol-water-acetic acid glacial (65:35:0.1)	Methanol-water-acetic acid glacial (65:35:0.1)
Flow rate	1.0 ml/min	1.0 ml/min	1.0 ml/min
Detection	270 nm	270 nm	270 nm

Preparation of standard solutions
The riboflavin was prepared by adding 20 mg riboflavin in 200 ml deionised water with the addition of three drops of pure acetic acid glacial. It was warmed at 80°C in a water bath in order to dissolve the riboflavin to get the final concentration of the standard 100 µg/ml.

IV. RESULTS AND DISCUSSION

Calibration curves for the standards (riboflavin) were obtained using a series of concentrations of these compounds at range of 1 to 10 microgram/ml. The regression coefficient is 0.998 as shown. Fig. 1,2,3,4 shows the chromatogram for the standard, extract sample for pulp, peel and seed respectively. The same separation conditions were employed for the analysis of the samples. The target compounds were identified using retention time match against those of the calibration standards. The wavelength was found to be 270 nm from UV.

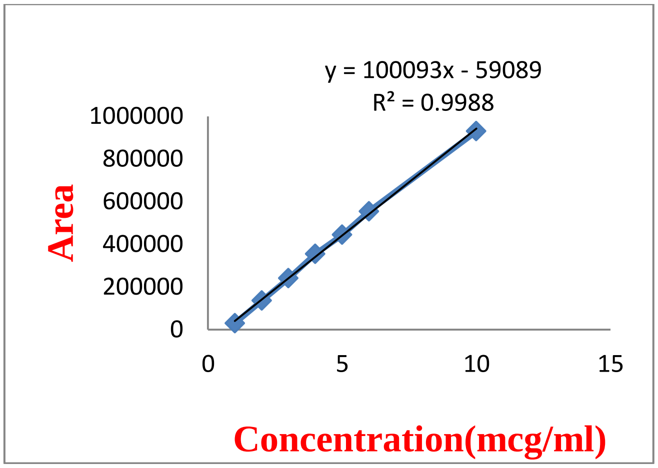

Fig 2: Calibration curve for the Std sample

The method for estimation of riboflavin was determined with clean chromatograms such as that shown below:

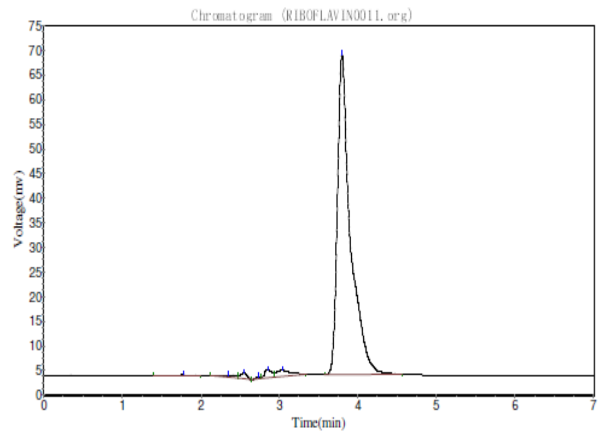

Fig 3: Chromatogram for std sample

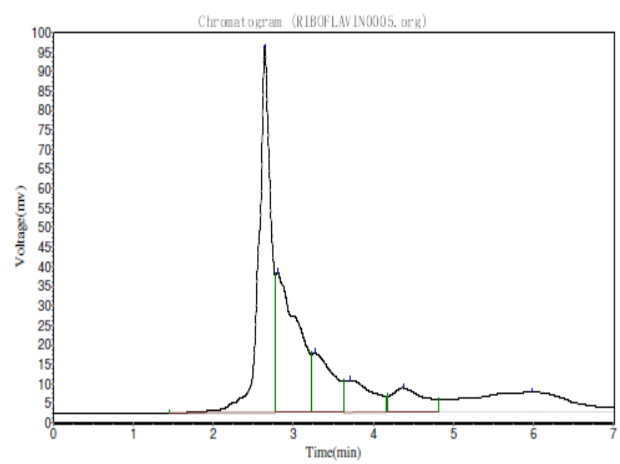

Fig 4: Chromatogram for peel extract

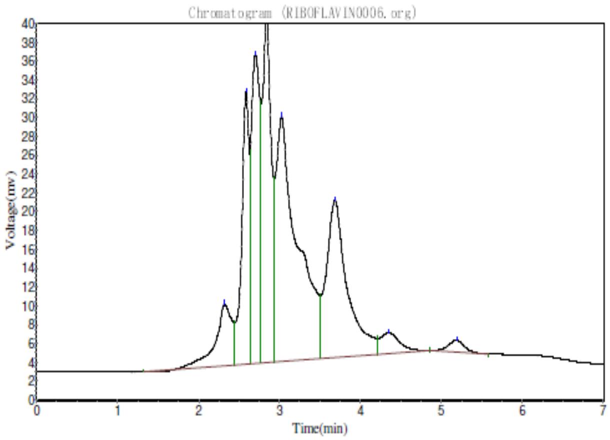

Fig 5: Chromatogram for seed extract

V. CALCULATION

1. The amount of riboflavin is present in the pulp is found be :

a.**E**xtrapolation=6.1×25 =152.5mg/ml

b.Direct comparison method = Test Area/Std Area (571974/929735.688xa10) x std conc

=6.1×25

=152.5mg/ml

2. The amount of riboflavin is present in the peel is found be :

a. Extra polation =2.3×25 =57.5mg/ml

b.Direct comparison method=TA/SA×SC

=202085.6/929735.688×10

=2.3×25

=57.5mg/ml

3. The amount of riboflavin is present in the seed is found be :

a**.** Extra polation =3.6×25 =90mg/ml

b.Direct comparison method=TA/SA×SC

=30218.7/929735.688×10

=3.6×25 =90mg/ml

VI. CONCLUSION

A simple, rapid, precise and accurate isocratic reversed-phase high performance liquid chromatography (HPLC) method was developed to determine the riboflavin in pulp, peel and seeds of Pumpkin and found to be 152.25mg/ml, 57.5mg/ml and 90mg/ml respectively both by extrapolation and direct comparison method. Separation was achieved using a 25 x 4.6 mm column, particle size 5 micron C_{18} reverse phase column (Phenomenex), with a mobile phase consisting of methanol, water and 0.01% CH_3COOH, in isocratic elution mode with a mobile phase flow rate of 1 ml/min, using UV visible detection at 270 nm. Regression analysis showed a good linear relationship $r^2 = 0.998$.

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