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HPLC analysis of β-sitosterol in herbal medicine and vegetable oils

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Abstract

Natural products have been considered anecdotal to the effective maintenance of good health. *Solal Beta sitosterol Capsules* is a herbal medicine and β-sitosterol is one of its main components. β-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health and enhance immunity in the human body. β-sitosterol can also be found in vegetable oils such as: wheat germ oil, cotton seed oil and so on. The amounts of β-sitosterol in herbal medicines and vegetable oils have not been reported in the literature since an analytical method has not yet been well established. This paper shows that high performance liquid chromatography (HPLC) is a suitable analytical method for determining β-sitosterol levels in *Solala beta sitosterol capsules and* several kinds of vegetable oils. HPLC chromatogram of β-sitostero standard and *solal betasitosterol capsules* was performed in the mobile phase as 100% Acetonitrile at pH 6.5 and showed retension time of 67.25 and 67.89 repectively.,95% Acetonitrile and 5% ethanol showed retension time for std beta sitosterol was 55.75,and 85% Acetonitrile and 15% ethanol showed retension time of 36.23 and 36.81 repectively .And Finally The amounts of β-sitosterols of the vegetable oils was also detected by HPLC showing retention time for wheat germ oil is 36.91 ,Cotton seed oil is 36.21 Soya been oil is 36.47 m,Peanut oil is 36.12. Thus this paper gives pertinent conditions for using high performance liquid chromatography (HPLC) to determine the β- sitosterol levels in herbal medicine as well as vegetable oils.

Key-Words: *Solal Beta sitosterol Capsules*, β-sitosterol, HPLC, Vegetable oils

Introduction

Natural products have been considered anecdotal to the effective maintenance of good health. β -sitosterol is a known plant sterol. The sterol in plant is called phytosterols. It is a waxy substance which is white in color. β -sitosterol has also been reported to be abundant in wheat germ oil, cotton seed oil, corn oil, and soybean oil[1]. Its efficacy of is reported as follows in the literature review. The structures of β -sitosterol and cholesterol are quite similar. It is reasonable that β-sitosterol can inhibit the absorbing of cholesterol in the body[2] and thus reduce the cholesterol levels in the plasma[3]. The liver function activity (GDP, GOP) can be improved with β-sitosterol[4], and this can reduce prostate cancer and colon-cancer cell growth[5,6], too. β-sitosterol can also be found in vegetables and fruits. The presence of β-sitosterol in soybean foods has been reported to suppress carcinogenesis. It can also be the factor used to form the lympho cells and NK in the immunity process circulation[7] β-sitosterol can be found in vegetables such as peanut oil.

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It is used in experiments for treating breast cancer and prostate cancer β-sitosterol in soybean oil has been reported to lower cholesterol levels[8]. β-sitosterol in corn oil, rice bran oil and other vegetables oil can affect the cholesterol level in the plasma[9]. Sterol and stanol in plants have been analyzed using high performance liquid chromatography atmospheric pressure chemical ionization mass spectroscopy (HPLC-APCI-MS)[10,11].

The literature survey reveals that Kalo et al. have used thin layer chromatography (TLC) to analyze triacylglycerol[12]. Kuksis et al. used gas chromatography (GC) to analyze sterol in plasma[13]. Kamm analyzed sterol in the cocoa cream with GC method in 2001[14] . Xin Zhang used capillary gas chromatography-mass spectrometry (GCMS) method to analyze β-sitosterol oxides in vegetable oils[15]. Billheimer used reversed-phase liquid chromatography to separate sterol esters[16]. Parcerisa analyzed olive oil using HPLC[17] and Kuksis applied the method of HPLC to analyze the plasma lipids too[18]. High-performance liquid chromatography (HPLC) methods have been widely used in the literature given above, but it has not

been exploited for analyzing β-sitosterol in herbal medicines and vegetable oils.

Material and Methods

Apparatus and reagents

The chromatographic system includes a Agilent MODEL gradient pump, a stainless steel injector (5 µL loop), and a UV-VIS detector operated at 198 nm for detecting β- sitosterol extracted from wheat germ oil, cotton seed oil, peanut oil , *Solal Beta sitosterol Capsules* and Standard β-sitosterol. RP-C-18 column was used as the analytical column. The optimal composition of the mobile phase is 15% Ethanol and 85% Acetonitrile. The flow rate of the mobile phase was 1 ml/min and the column temperature was kept at 25°C. The sample solution and reagent solution were degassed before each run Unless otherwise specified, all reagents were HPLC grade and these include: methanol, ethanol, acetonitrile, and potassium hydroxide. Reagents were degassed in an ultrasonic bath as required before injecting into the HPLC.

Sample preparation

Wheat germ oil, cotton seed oil, peanut oil, soy bean oil, were obtained from a supermarket in the sangli. *Solal Beta sitosterol Capsules* obtained from local market of sangli.. All samples were filtered as required before injecting into HPLC. An authentic chemical sample of β- sitosterol was purchased from Sigma-Aldrich Co. (U.S.A) and a concentration of 1mg/1ml was prepared by dissolving it in chloroform.

Results and Discussion

The high performance liquid chromatography (HPLC) is a suitable analytical method for determining β-sitosterol levels in *Solala beta sitosterol capsules and* several kinds of vegetable oils. HPLC chromatogram of β-sitosterol standard and *solal betasitosterol capsules* was performed in the mobile phase as 100% Acetonitrile at pH 6.5 and showed retension time of 67.25 and 67.89 repectively(Fig no 1), 95% Acetonitrile and 5% ethanol showed retension time for std beta sitosterol was 55.75(Fig no 2), and 85% Acetonitrile and 15% ethanol showed retension time of 36.23 and 36.81 repectively(Fig no 3). And Finally the amounts of β-sitosterols of the vegetable oils was also detected by HPLC showing retention time for wheat germ oil is 36.91 ,Cotton seed oil is 36.21 Soya been oil is 36.47 ,Peanut oil is 36.12(Table no 1).

This paper gives the pertinent conditions for using high performance liquid chromatography (HPLC) to determine the β- sitosterol levels in salol beta sitosterol capsules. This analytical method can also be applied to determine the amounts of β- sitosterol in vegetable oils.

References

1. Chen RR (1991). Plant oil. Biomedicine, Taipei Publishers, Taiwan: 283-287.
2. Tatu A, Miettinen A, Helena G (2002). Ineffective decrease of serum cholesterol by simvastatin in a subgroup of hypercholesterolemic coronary patients. Atherosclerosis 164: 147-52.
3. MacLatchy DL, Van Der Kraak GJ (1995). The phytoestrogen betasitosterol alters the reproductive endocrine status of goldfish. Toxicol Appl. Pharmacol. 134: 305-12.
4. Zak A, Vecka M, Tvrzicka E, Hruby M, Novak F, Papezova H, Lubanda H, Vesela L, Stankova B (2005). Composition of Plasma Fatty Acids and Non-Cholesterol Sterols in Anorexia Nervosa. Physiol. Res. 54: 443-51.
5. Awad AB, Fink CS (2000). Phytosterols as anticancer dietary components: evidence and mechanism of Action 1,2. J. Nutr. 130: 2127-30.
6. Awad AB, Chan KC, Downie AC, Fink CS (2000). Peanuts as a source of beta-sitosterol, a sterol with anticancer properties. Nutr. Cancer. 36: 238-41.
7. Bouic PJD, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP (1996). Beta-sitosterol and Beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: Implications for their use as an immunomodulatory vitamin combination. Int. J. Immunopharmac. 18: 693-700.
8. Cicero AF, Fiorito A, Panourgia MP, Sangiorgi Z, Gaddi A (2002). Effects of a new soy/beta-sitosterol supplement on plasma lipids in moderately hypercholesterolemic subjects. J. Am. Diet Assoc. 102: 1807-11.
9. Frank N, Andrews FM , Elliott SB , Lew J, Boston RC (2005). Effects of rice bran oil on plasma lipid concentrations, lipoprotein composition, and glucose dynamics in mares. J. Anim. Sci. 83: 2509-18.
10. Burkhardt MR, ReVello RC, Smith SG, Zaugg SD (2005). Pressurized liquid extraction using water isopropanol coupled with solid-phase extraction cleanup for industrial and anthropogenic waste-indicator compounds in sediment. Analytica Chimica Acta. 534: 89–100.
11. Mezine I, Zhang H, Macku C, Lijana R (2003). Analysis of plant sterol and stanol esters in cholesterol-lowering spreads and

beverages using high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectroscopy. J. Agric. Food Chem. 51: 5639-46.
12. Kalo P, Kuuranne T (2001). Analysis of free and esterified sterols in fats and oils by flash chromatography, gas chromatography and electrospray tandem mass spectrometry. J. Chromatogr. 935: 237- 42.
13. Kuksis A, Marai L, Myher JJ (1991). Plasma lipid profiling by liquid chromatography with chloride-attachment mass spectrometry. Lipids 26: 240-44.
14. Kamm W, Dionisi F, Fay LB, Hischenhuber C, Schmarr HG, Engel KH (2001). Analysis of steryl esters in cocoa butter by on-line liquid chromatography-gas chromatography. J. Chromatogr. A. 918: 341- 45.
15. Zhang X, Diane JD, Miesch M, Geoffroy P, Raul F, Roussi S, Dalal AW, Marchioni E (2005). Identification and quantitative analysis of sitosterol oxides in vegetable oils by capillary gas chromatography– mass spectrometry. Steroids 6116: 1-11.
16. Billheimer JT, Avart S, Milani BJ (1983). Separation of steryl esters by reversed-phase liquid chromatography. Lipid Res. 24: 1646-52.
17. Parcerisa J, Casals I, Boatella J, Codony R, Rafecas MJ (2000). Analysis of olive and hazelnut oil mixtures by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry of triacylglycerols and gas-liquid chromatography of non-saponifiable compounds (tocopherols and sterols). Chromatogr. A. 881: 149-54.
18. Kuksis A, Myher JJ, Marai L, Little JA, McArhur RG, Roncari DA (1986). Fatty acid composition of individual plasma steryl esters in phytosterolemia and xanthomatosis. Lipids 21: 371-77.

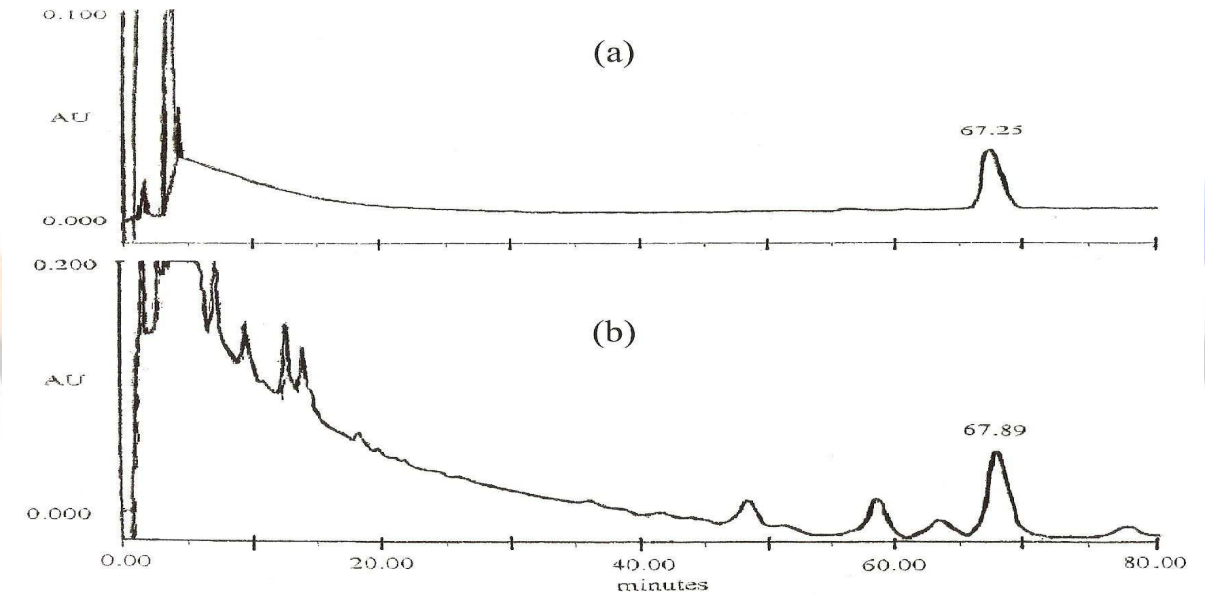

Fig. 1: HPLC chromatogram of β-sitosterol standard and *solal betasitosterol capsules* was performed in the mobile phase as 100% Acetonitrile at pH 6.5 and showed retension time of 67.25 and 67.89 repectively

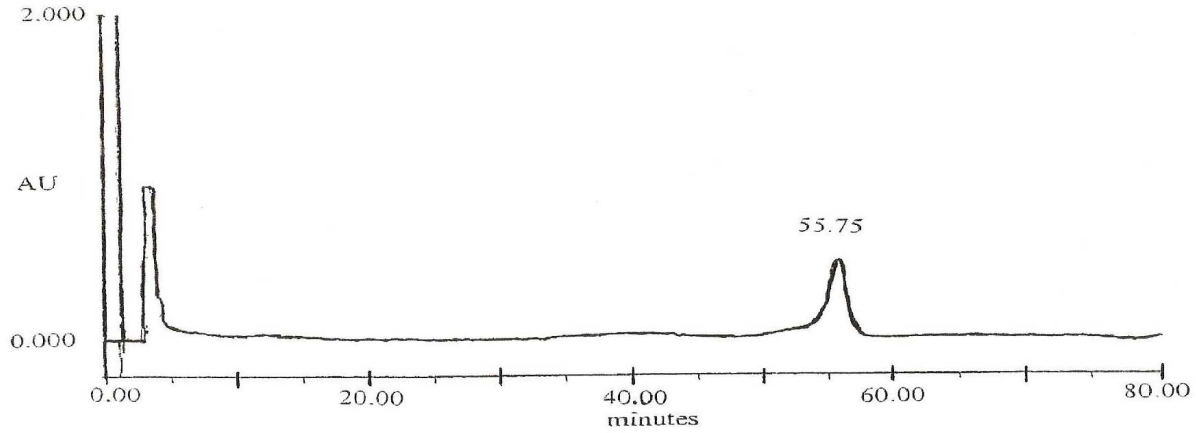

Fig. 2: HPLC chromatogram of β-sitosterol standard was performed in 95% Acetonitrile and 5% ethanol and showed retension time was 55.75

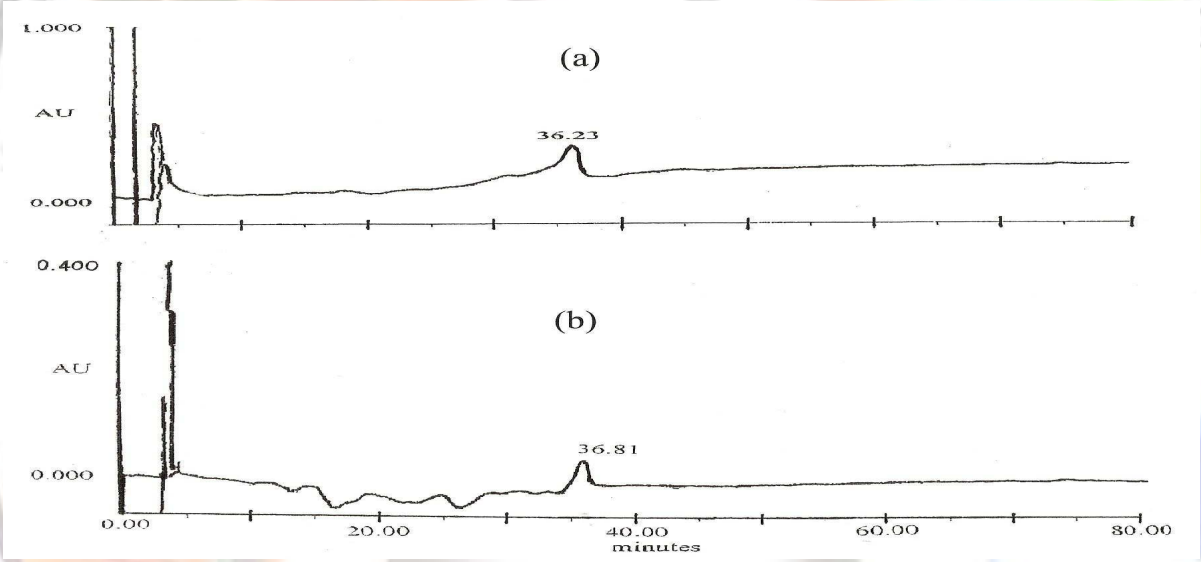

Fig. 3: HPLC chromatogram of β-sitosterol standard and *solal betasitosterol capsules* was performed in the mobile phase 85% Acetonitrile and 15% ethanol showed retension time of 36.23 and 36.81 repectively .

Table 1: The Retension time of β-sitosterols of the vegetable oils detected by HPLC.

S/No.	Vegetable oil	Retension time
1.	Wheat germ oil	36.91
2.	Cotton seed oil	36.21
3.	Soya been oil	36.47
4.	Peanut oil	36.12
5.	*solal betasitosterol capsules*	36.81
6.	β-sitosterol standard	36.23