Vol 3 (1), June 2015; 39-44

ISSN 2311-4673
Journal of Pharmacy and Pharmaceutical Sciences
(Volume 3, Issue 1, 2015)

Quantitive Evaluation of ß -Sitosterol with Hplc in Saw Palmetto Fruits and Seeds

Saleha Suleman Khan[*1], *Zeeshan Ahmed Sheikh*[1], *Aqib Zahoor*[1], *Sadia Shakeel*[2,3], *Khan Usmanghani*[1,2]

[1]Herbion Pakistan (Pvt.) Ltd., Karachi, Pakistan
[2]Faculty of Pharmacy, Jinnah University for Women Karachi. - Sind - 74600 – Pakistan
[3]Dow College of Pharmacy, Dow University of Health Sciences, Karachi. Pakistan

ABSTRACT

The technological advancements in the processes of isolation, purification and structural elucidation of natural compounds have made it probable to generate appropriate strategies for the analysis of quality and the standardization of plant based medicines. The current study was conducted to evaluate the biomarker ß-sitosterol in seeds and fruits of Saw palmetto by HPTLC. Quantitative analysis of ß-sitosterol was conducted; using silica gel 60 F254 coated plates as a stationary phase to augment the identification and determination of ß-sitosterol component by using petroleum ether and ethyl acetate 8:2 (v/v) as a solvent system. The Rf value (0.58) of ß-sitosterol in reference standard and both fruits and seeds was found comparable under UV light at 450 nm. An accurate, rapid and simple HPTLC method for quantitative estimation of biomarker ß-sitosterol in fruits and seeds of plant Saw palmetto has been developed. The present standardization provides a specific and rapid tool in the herbal research, permitting to set quality specifications for identity, transparency and reproducibility of biomarker ß-sitosterol.

Keywords: ß-sitosterol, HPTLC method, Quantitative analysis.

INTRODUCTION

Plants have been the persistent source of acquiring diverse therapeutically active compounds. Various forms of botanically derived materials have been used to treat innumerable ailments. These materials have usually been in the form of powders made from one or more plants or plant parts or extracts derived from whole plants or selected plant parts. These powders and extracts are complex mixtures of both biologically active and inactive compounds. The complex chemical nature of the botanical materials makes it complicated to use them in conventional manner. This probable variation in the chemical composition of different batches of material obtained from different plant harvests makes such materials unsuitable for use in quantifiable situations[1].

The problem allied with the intrinsic chemical intricacy of herbal medicaments has resulted in a great deal of effort intended for the separation and isolation of the biologically active components from numerous medicinally important plants. The technological advancements in the processes of isolation, purification and structural elucidation of natural compounds have made it probable to generate appropriate strategies for the analysis of quality and

*Corresponding author: saleha.khan@herbion.com

the standardization of plant based medicines [2]. An appliance of highly oriented hyphenated techniques provides a definite tool in herbal investigations. A variety of sophisticated methods such as spectrophotometric, chromatographic, polarography, electrophoresis, and the use of molecular biomarkers in fingerprints are presently employed in standardization of plant based medicines. TLC and HPTLC fingerprint profiles are used for ensuring the identity, transparency and potency of the herbal formulations [3]. TLC is the common fingerprint method that is commonly used for the evaluation of stability and consistency of polyherbal preparations from different manufactures[4]. HPTLC fingerprint is mostly used for evaluating the compounds with low or moderate polarities [5]. Combined chromatographic fingerprinting with metabolomics facilitates to control the intrinsic quality of herbal drugs[6]. The innovation of analytical techniques provides a specific and rapid tool in the herbal research, permitting to set quality specifications of plant based medicines. Once isolated and purified, the various active components are used in clinical settings to establish the medicinal effectiveness of a specific component. The suspected active component is typically mixed with a pharmaceutically suitable carrier and subjected to further studies in laboratory animals and eventual clinical trials in humans. Upon proof of clinical efficacy, these types of drugs are considered to be pharmaceutical grade because they enclose a single, or at most a small number of, well-characterized compounds which are present in known quantities[7]. Therefore the present study was directed towards the quantitative estimation of biomarker ß-sitosterol (Figure 1) in fruits and seeds of plant Saw palmetto by employing HPLTLC.

EXPERIMENTAL

Material and Method: Petroleum ether, ethyl acetate (Merck, Pakistan). Methanol and ethanol of analytical reagent grade (Merck, Darmstadt, Germany). ß-sitosterol reference standard (Sigma-Aldrich GmbH, Germany). All other solvents and chemicals were of the highest analytical grade and used as supplied.

Apparatus: Spotting device: Linomat V Automatic Sample Spotter (CAMAG, Muttenz, Switzerland), Syringe: 100 µl (Hamilton, Bonaduz, Switzerland),Thin layer chromatographic (TLC) chamber: Glass twin trough chamber (20 ? 10 ? 4c m) (CAMAG),Densitometer: TLC Scanner 3 linked to Win Cats software (CAMAG),HPTLC plates: 20 ? 10 cm, 0.2 mm thickness pre-coated with silica gel 60 F 254 (Merck)

Experimental conditions: Temperature 25±2ºC; relative humidity 40%

Quantitative Estimation of ß-sitosterol by HPTLC-Densitometry

Standard Preparation: The standard solution was prepared containing known concentration of 0.3 mg/ml by dissolving 3 mg standard of ß-sitosterol in 10 ml of methanol.

Sample Preparation: Approximately 5.0 g of capsule powder was weighed accurately in to 250 ml of round bottom flask. 50 ml mixture of petroleum ether and ethyl acetate (8:2) was added and was refluxed for 30 min. on heating mental. The mixture was cooled at room temperature and the solvent was decanted and filtered through Whatman filter paper No.44. Process was repeated four times more (50x3).The filtrate was collected into the same round-bottomed flask. The organic fraction was evaporated under vacuum. The dry residue was dissolved in 10 ml of methanol and transferred into a 10 ml volumetric flask. The sample was sonicated until the complete dissolution of residue.

Procedure:

TLC Preparation: Analysis was performed on 10 x 10 cm HPTLC silica gel G60F254 plates with fluorescent indicator. Before starting the analysis, HPTLC plate were cleaned by predevelopment with methanol by ascending method. (HPTLC Plate was immersed in a CAMAG glass chamber (20 x 10 cm), containing 30 ml methanol (HPLC grade) as solvent system. The chamber was covered with glass lid and

left till development of the plate to the top with methanol. After complete development, the plate was removed from TLC glass chamber and dried in an oven at 105? C for 5 min).

Application Procedure: Three spots of 10 µl were applied (in the form of band) of standard preparation along with three spots of 10 µl of sample preparation as the bands on the same plate by means of a CAMAG Linomat 5 (automated spray-on applicator equipped with a 100 µl syringe and operated with the settings band length 6 mm, distance between band 15 mm, distance from the plate side edge 15 mm, and distance from the bottom of the plate 15 mm).

TLC Development: The plate was developed by immersing sample HPTLC plate in a CAMAG glass chamber (20 x 10 cm) containing the solvent system petroleum ether and ethyl acetate 8:2 (v/v). Wait to develop the plate to a distance of 8 to 9cm. After complete development, the plate was allowed to dry by keeping in fume cupboard for 10 minutes and then kept in hot air oven for 5 min at 105 °C. TLC plate was sprayed with anisaldehyde /sulfuric acid reagent. Violet spots (Figure 2) appeared after drying the TLC plates in hot air oven for 5 min at 105 °C.

Composition of anisaldehyde/sulfuric acid reagent: 0.5 ml of anisaldehyde was dissolved in 9 ml of 96%ethanol; 0.5 ml of 97%H2SO4 and 0.1 ml of acetic acid was added and mixed.

TLC Scanning: The plate was scanned in the densitometer by linear scanning at 450 nm for ß-sitosterol by using a TLC Scanner III CAMAG with a D2 source, and integrate the area of the spots (Figure 3) corresponding to ß-sitosterol standard. Amount of ß-sitosterol in seeds and fruits of saw palmetto was calculated by following formula:

ASMP x WSTD x f x Dilution of Smp x application vol. of sample × P x D x 10

ASTD x Dilution of Std x WSMP x application of vol. standard x 100

ASMP = Avg. Area of Sample,

ASTD = Avg. Area of Standard

WSTD = Weight of Standard, mg

WSMP = Weight of Sample, g

Dilution of Smp = Dilution of Sample, ml

Dilution of Std = Dilution of Standard, ml

P = Percent Purity of Standard

RESULTS:

Saw Palmetto seeds Powder = 1199.7 × 1.3 × 10 × 90 1507.4 × 5.000 × 10 × 100 = 0.186 mg/5gm

Saw Palmetto fruit Powder = 877.5 × 1.3 × 10 × 90

[1507.4 × 5.000 × 10 × 100 = 0.1362 mg/5gm

The current study was conducted to evaluate the biomarker ß-sitosterol in seeds and fruits of Saw palmetto. Quantitative analysis of ß-sitosterol was conducted; using silica gel 60 F254 coated plates as a stationary phase to augment the identification and determination of ß-sitosterol components by using petroleum ether and ethyl acetate 8:2 (v/v) as a solvent system. After developing and drying, the plates were observed under UV light for the presence of ß-sitosterol, which was detected by prominent violet color spot. The Rf value (0.58) of ß-sitosterol in reference standard (Figure 3) and both fruits and seeds (Figure 4, 5) was found comparable under UV light at 450 nm.

DISCUSSION

Saw palmetto (Serenoarepens) is a native plant of North America, commonly found in Florida[8]. Saw palmetto contains many biologically active chemicals. The berries of the saw palmetto plant have been used

for medicinal purposes for centuries. Both berry raw material and extract are found in dietary supplements and are most commonly used to treat symptoms related to benign prostatic hyperplasia (BPH)[9]. Saw palmetto berries are a tonic herb that is used in the treatment of debility, urinary tract problems and for reducing enlarged prostate glands. The partially dried ripe fruit is aphrodisiac, urinary antiseptic, diuretic, expectorant, sedative and tonic[10]. Saw palmetto is one of the few herbs that are considered to be anabolic. The fruit pulp, or a tincture, is given to those suffering from wasting disease, general debility and failure to thrive. The fruit also has a beneficial effect on the urinary system, helping to reduce the size of an enlarged prostate gland and strengthening the neck of the bladder[11]. The fruit has a probable estrogenic action, it is prescribed in the management of impotence, reduced or absent sex drive and testicular atrophy in men and to stimulate breast enlargement in women. The fruit is also used in the treatment of colds, coughs, irritated mucous membranes, asthma etc. A suppository of the powdered fruits, in cocoa butter, has been used as a uterine and vaginal tonic[12].

In conjunction with an AOAC (Association of Analytical Communities) Task Force on Dietary Supplements, a method was validated for measurement of 3 plant sterols (phytosterols) namely campesterol, stigmasterol, and beta-sitosterol in saw palmetto raw materials, extracts, and dietary supplements[13]. ß-sitosterol is an ancient molecules in plants kingdom. The structures of ß -sitosterol and cholesterol are quite similar. It is rational that ß-sitosterol can reduce the absorbing of cholesterol in the body thereby decrease the cholesterol levels in the plasma[14]. Literature review revealed that ß-sitosterol has been isolated and purified by different chromatographic methods from diverse plant families [10, 15]. In current study quantitative estimation of the biomarkers ß-sitosterol in seeds and fruits of Saw palmetto was evaluated by HTLC using petroleum ether and ethyl acetate 8:2 (v/v) as a solvent system. Sample preparation and development of appropriate mobile phase are two imperative stages in analytical procedures, which becomes more considerable for plant based medicines owing to their complexity of the chemical compounds and their affinity towards different solvent systems[16]. The Rf value (0.58) of ß-sitosterol in reference standard and both fruits and seeds was found comparable under UV light at 450 nm. HPTLC method was found to be simple, reliable, and convenient for routine analysis. Other researchers have also employed HPTLC for the determination of ß-sitosterol. Its mean advantages include its simplicity, accuracy and selectivity [17-19].

CONCLUSION

An accurate, rapid and simple HPTLC method for quantitative estimation of biomarker ß-sitosterol in fruits and seeds of plant Saw palmetto has been developed. The present standardization provides a specific and rapid tool in the herbal research, permitting to set quality specifications for identity, transparency and reproducibility of biomarker ß-sitosterol.

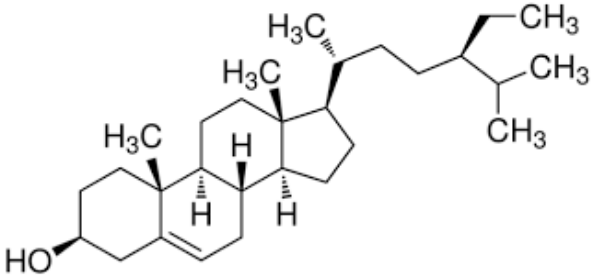

Figure 1: Structure of ß-sitosterol

Figure 2: TLC image of ß-sitosterol in fruits and seeds

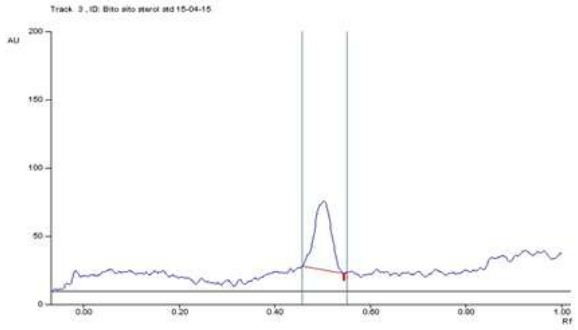

Figure 3: Peak response of ß-sitosterol standard

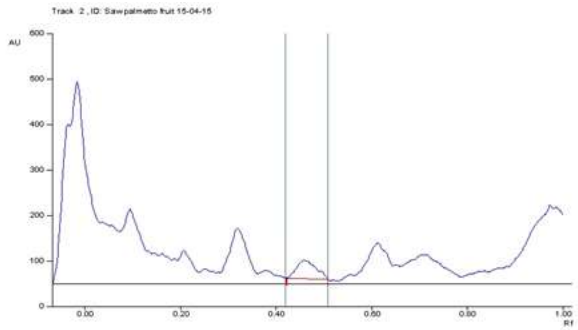

Figure 4: Peak response of ß-sitosterol in fruits

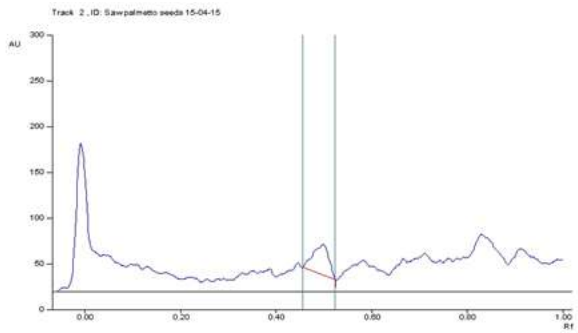

Figure 5: Peak response of ß-sitosterol in seeds

REFERENCES

1. Bedner M, Schantz MM, Sander LC, Sharpless KE. Development of liquid chromatographic methods for the determination of phytosterols in Standard Reference Materials containing saw palmetto. Journal of Chromatography A. 2008;1192(1):74-80.

2. Calixto BJ. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res. 2000;33:2.

3. Pattanaya P, Jena RK, Panda SK. HPTLC fingerprinting in the standardization of sulaharan yoga: An ayurvedic tablet formulation. Int J Pharm Sci Rev Res. 2010;3(2):33-6.

4. Zhang Z, Liang Y, Xie P, Chau F, Chan K. Chromatographic Fingerprinting and Chemometric Techniques for Quality Control of Herb Medicines. Data Analytics for Traditional Chinese Medicine Research: Springer; 2014. p. 133-53.

5. Yongyu Z, Shujun S, Jianye D, Wenyu W, Huijuan C, Jianbing W, et al. Quality control method for herbal medicine-chemical fingerprint analysis. Quality Control of Herbal Medicines and Related Areas InTech. 2011:171-94.

6. Zhong L, Hu D, Xu H, Han Q, Bian Z. Efficacy-Driven Quality Control Platform for Chinese Herbal Medicine. Pharm Anal Acta. 2014;5(291):2.

7. Catchpole O, Perry N, Da Silva B, Grey J, Smallfield B. Supercritical extraction of herbs I: Saw Palmetto, St John's wort, Kava root, and Echinacea. The Journal of supercritical fluids. 2002;22(2):129-38.

8. Preuss HG, Marcusen C, Regan J, Klimberg IW, Welebir TA, Jones WA. Randomized trial of a combination of natural products (cernitin, saw palmetto, B-sitosterol, vitamin E) on symptoms of benign prostatic hyperplasia (BPH). International urology and nephrology. 2001;33(2):217-25.

9. Northern V, Southwest V. Saw Palmetto.

10. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol-a review. Eur J Med Plants. 2014;4:590-609.

11. Wilt TJ, Ishani A, MacDonald R, Stark G, Mulrow CD, Lau J. Beta-sitosterols for benign prostatic hyperplasia. The Cochrane Library. 1999.

12. Lowe FC, Ku JC. Phytotherapy in treatment of benign prostatic hyperplasia: a critical review. Urology. 1996;48(1):12-20.

13. Kelly JP, Kaufman DW, Kelley K, Rosenberg L, Mitchell AA. Use of herbal/natural supplements according to racial/ethnic group. Journal of Alternative & Complementary Medicine. 2006;12(6):555-61.

14. Ye J-C, Chang W-C, Hsieh DJ-Y, Hsiao M-W. Extraction and analysis of ß-sitosterol in herbal medicines. J Med Plants Res. 2010;7:522-7.

15. Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Progress in lipid research. 2002;41(6):457-500.

16. Shahare M, D'Mello P. Standardization of Bacopa Monnieri and its formulations with reference to Bacoside A, by high performance thin layer chromatography. Tablet. 2010;1000:1000.

17. Patel DK, Patel K, Dhanabal S. Phytochemical standardization of Aloe vera extract by HPTLC techniques. Journal of Acute Disease. 2012;1(1):47-50.

18. Andola H, Rawal R, Rawat M, Bhatt I, Purohit VK. Analysis of berberine content using HPTLC fingerprinting of root and bark of three Himalayan Berberis species. Asian J Biotechnol. 2010;2(4):239-45.

19. Patel DK, Patel K, Dhanabal S. Standardization of Berberis aristata extract through conventional and modern HPTLC techniques. Asian Pacific Journal of Tropical Disease. 2012;2:S136-S40.