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"A COMPREHENSIVE EXAMINATION OF ANALYTICAL TECHNIQUES FOR β - SITOSTEROL"

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ABSTRACT

Phytosterols are bioactive compounds that are naturally present in plant cell membranes with a similar chemical structure to the mammalian cell- derived cholesterol. β-sitosterol stands out as the primary phytosterol compound abundant in plants. The reason it is present in so many plant and vegetable oils is due to its steroidal lipophilic nature. From various in-vitro and in vivo studies it is found to possess important activities as Anticancer, Antioxidant, Antimicrobial, immunomodulatory, Anti-inflammatory, lipid lowering, hepato-protection. A properly developed and validated method of quantification can be used for phytochemical profiling and estimation of the standard compound. Analysts are able to improve their comprehension and tailor their own experimental strategies for drug analysis by thoroughly evaluating the procedures used in earlier research. Analysts who are looking for information on the precise set of instruments and solvents used in the analysis of beta sitosterol may find this review useful. Thus article aims to exhaustively explore analytical methods for β-sitosterol, encompassing methods such as GC-MS, HPLC, and HPTLC.

Key words: Beta sitosterol, Analytical methods, HPLC, HPTLC, GC-MS, HSCCC.

Introduction

The word "phytochemicals," which refers to substances derived from plants, was first used in 1994 and quickly gained popularity among scientists and researchers. Among these substances are phytosterols, which are a significant class of bioorganic compounds and a subgroup of steroids. With a structural resemblance to cholesterol, phytosterols are found in a wide variety of flora, animals, and fungus. In the physiology of eukaryotic organisms, phytosterols are vital components. For example, in mammals, cholesterol makes up the majority of the cellular membrane, which influences the membrane's fluidity and functions as a secondary messenger in embryonic signalling. The primary advantage of these naturally occurring metabolites is in their inclusion as components of natural meals that are known to promote health.[1]

The three main phytosterols in human herbal nutrition are β-sitosterol, campesterol, and stigmasterol, which make up 65%, 30%, and 3% of the diet, respectively. With a lengthy history of use in food and medicine, phytosterols are widely acknowledged for considered safe (GRAS), and there have been no reports of any negative side effects.

Natural phytosterols are called β-Sitosterol. It is a steroidal substance derived from plants that resembles cholesterol.Its steroidal lipophilic character explains why it's found in numerous plant and vegetable oils. Scientists were intrigued by it because it might have an impact on the absorption of cholesterol. It was believed to lower the risk of cardiovascular illnesses as a result.[2]

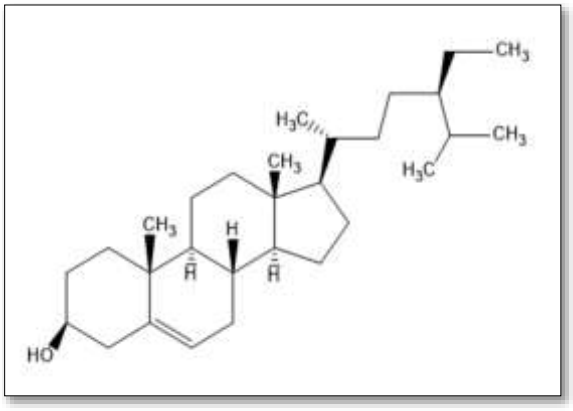

Fig. 01 Chemical structure of Beta sitosterol

Table 1. Drug profile	
NAME	Beta-sitosterol
IUPAC NAME	17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
MOLECULAR	$C_{29}H_{50}O$

FORMULA	
MOLECULAR WEIGHT	414.718 $g \cdot mol^{-1}$
MELTING POINT	136 to 140 °C
PHYSICAL DESCRIPTION	Solid
CATEGORY	Anti-cancer, sedative effects, analgesic, immunomodulatory, antimicrobial, anti – inflammatory, lipid lowering effect, hepatoprotective, protective effect against NAFLD and respiratory diseases, wound healing effect, antioxidant and anti-diabetic activities**.**
SOLUBILITY	Insoluble in water and Acetonitrile, soluble in ethanol, ethyl acetate and Methanol

Sources of β-sitosterol

Out of the 200 plant sterols found in plants, β-sitosterol makes up the majority (60%) of phytosterols, with campesterol accounting for 24%, stigmasterol for 7%, and smaller amounts of 5-avenasterol, brassica sterol, beta-sitosterol, and campestanol. EFSA (2009) states that a daily dose of 1.5 to 2.4 g/kg of phytosterols can averagely lower blood levels of low-density lipoproteins by 7 to 10.5%.

β-sitosterol is a white, waxy material found in a range of naturally occurring plant sources, including cereals, pulses, legumes, vegetables, and fruits. Supercritical fluid extraction, traditional solvent extraction methods, and other chromatographic techniques are some of the technologies used to extract β-sitosterol. Pistachios have the greatest β-sitosterol concentration of any nut. They are produced from acetyl co-A and , because of their structural resemblance to cholesterol, they stabilize the phospholipid bilayer in nuts. [3]

Pharmacological and biological activity

Beta sitosterol has various reported pharmacological activities like Anticancer activity (against Breast cancer, Prostate cancer, Fibrosarcoma, Uterine, cervix cancer etc), Hormonal, Antioxidant, Angiogenic, Antimicrobial, Anti-inflammatory, Immuno-modulatory, Antihyperlipidemic activity, Antiatherosclerosis, Central nervous system, Antidiabetic, Neurodegerative disease, Liver function, GI system, Reproductive system, Hypersensitivity.[4]

Analytical methods

Determining the drug in different biological samples is crucial for drug development and discovery since it gives pharmacokinetic information and is required to calculate dose and safety limits. Because the phytoconstituents in herbal medications are complex, quantifying their bioactive indicators is a difficult undertaking. To guarantee optimal pharmacological

efficacy, it is preferable to quantify the active ingredient. For herbal formulations to be standardized, analytical procedures for the analysis of phytoconstituents must be developed.

The techniques for separating and quantifying beta-sitosterol by high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), GC, and MS techniques have been compiled in this review.[5]

Beta Sitosterol Analysis Using Hplc Technique

Currently, high-performance liquid chromatography (HPLC) stands out as one of the most powerful tools in analytical chemistry. All substances present in a sample that can dissolve in a liquid can be separated, identified, and quantified using this technique. For both quantitative and qualitative analysis of pharmacological products, high performance liquid chromatography (HPLC) is the most accurate and efficient analytical technique. [6]

The HPLC system consists of an injector, column, detector, integrator, pump, and display system. The characteristics of HPLC technology include the following.

Greater resolution; small diameter ; stainless steel; glass column; quick analysis; greater pressure and regulated flow rate in the mobile phase.

The HPLC is classified into the following types based on the stationary phase-Mobile phase used.

Normal Phase HPLC: In this type stationary phase is polar. Silica is the most common material utilized, while hexane, chloroform, and diethyl ether are used as non-polar phases. Polar samples are typically analysed in it.

Reverse Phase HPLC: Here, the stationary phase is hydrophobic or non-polar, and the mobile phase is polar. It is reverse of normal phase HPLC. The more non-polar a compound is, the longer it will spend on the column because of non-polar stationary phase (usually made of a hydrocarbon chain bonded to the silica), and non-polar compounds interact more strongly with this stationary phase, leading to longer retention times. [7]

Table 02. HPLC methods

Sr No.	Phytoconstituents	Stationary phase	Mobile phase	Detector	Flow Rate (ml/min) and RT(min)	Ref no.
01	β-Sitosterol (Analysis and Extraction)	RP-18 column (Inertsil 7 ODS-3 4.6	Ethanol: ACN (15:85)	UV-VIS detector 198nm	1ml/min 36.23 min.	8

		mm i.d., 250 mm, Merck)				
02	β-Sitosterol (Phytosterols in Cissus quadrangularis)	Cosmosil C8 column (250 × 4.6 mm, 5 µm i.d.)	Acetonitrile: water (95:5 v/v)	UV-VIS detector 202nm	2.0ml/min 8.23min	9
03	β-sitosterol and gallic acid in Nigella Sativa seeds	C18 column (250 mm × 4.6 mm id, 5 µm particle size)	Acetonitrile: water 60:40 (v/v) (0.05% OPA)	UV-VIS detector 210nm	0.5ml/min 9.58min	10
04	Lupeol, Stigmasterol and β-Sitosterol in Extracts of Adhatoda vasica Nees Leaves	RP-Phenomenex C18 (250mm×4.6 mm; 5µ) column	0.1%v/v formic acid in water and methanol (28:82%v/v)	PDA Detector 208nm	0.8ml/min 20.72 min	11
05	Stigmasterol and β-sitosterol in Manasamitra Vatakam-an ayurvedic herbomineral formulation	Phenomenex RP C18 column (250 x 4.6 mm; 5 µm)	Methanol and ACN (95:5% v/v)	PDA Detector 208nm	1.0ml/min 18.1min	12
06	β-Sitosterol analysis in supplement	LC Column 150 x 4.6 mm Phenomenex	Methanol and Acetonitrile (90: 10 v/v).	UV-VIS detector202nm	1.5 mL/min 13.24min	13
07	Linoleic Acid and Beta Sitosterol in Solanum nigrum	Acclaim® 120 A C18 column (4.0 mm × 250mm, 5µm,	0.05% phosphoric acid and acetonitrile 90:10(v/v)	PDA Detector 256nm	1.0ml/min 10.60min	14

08	Scopoletin And B-Sitosterol In Plant Extract Of Girardinia Diversifolia	Shimpack RP-C18 (50 mm*4.6 id, 5 µm)	Acetonitrile: water 85:15 %lv/v	UV detector347nm	1.3 ml/min 1.3min	15
09	β-Sitosterol isomers	Symmetry C18 Column (5µm, 3.9×150mm)	MeOH	UV detector210nm	1.0ml/min 21.80min	16

Beta Sitosterol Analysis Using GC and MS Technique

Gas chromatography-mass spectrometry (GC-MS) is a central analytical method used in many different fields and applications.

However, the relatively narrow range of volatile, thermally stable chemicals that are accessible to investigation presents GC-MS with a significant "Achilles heel." The restricted ability to analyse big compounds that are thermally labile and low-volatility significantly limits the utility and usability of GC-MS. [17]

Gas chromatography (GC)

Gas chromatography is a widely used chromatographic technique in analytical chemistry that is employed to separate and examine compounds that have the ability to evaporate without decomposing.

When separating complex mixtures of volatile compounds, it is the most practical and efficient procedure. An eluent, or gaseous mobile phase, is used in gas chromatography to transport the analyte through a column that has been packed or coated with a stationary phase. There are a few hundred-meter-long GC columns.

When using gas chromatography, the stationary phase is often a packing of minute, inert particles (such as diatomaceous earth) covered in a liquid that is either nonpolar or restricted to the interior surface of the column. There is a thin layer of 0.1–5 µM in this liquid. The mobile phase is an inert gas that does nothing more than push the analyte molecules down the column, like helium, nitrogen, or argon. [18]

Mass Spectriometry (MS)

Chemical species are ionized via mass spectrometry. It is an analytical technique that classifies the ions based on their mass-to-charge ratio. Using a mass spectrum, the masses in a sample are determined. Across a broad range of applications, mass spectrometry can be used on both pure

samples and complex mixtures. The three components of a mass spectrometer are an ion source, a mass analyser, and a detector. The ionizer converts part of the material into ions. [19]

Table 03. Beta sitosterol analysis using GC-MS

Sr No.	Phytoconstituents	Stationary phase	Carrier gas	Flow rate	Ref no.
01	Campesterol, Stigmasterol, and beta-Sitosterol in Saw Palmetto RawMaterials and Dietary Supplements	OV-1701 column (30m× 0.25mm×0.25μm)	He gas (99.99% purity),	-	20
02	β-Sitosterol	Column. —Capillary, split-mode, 25 m 0.32 mm 0.17 m film thickness, cross-linked 5% phenyl–methyl silicone or methyl silicone gum (e.g., Hewlett Packard No. HP-5, Ultra 2, or HP-1; Agilent Technologies).	hydrogen flame ionization detector	-	21
03	β-Sitosterol Betulinic Acid,(+) Eriodictyol,(+) Epipinoresinol, and Secoisolariciresinol , in Crude Extract and Ethyl Acetate Fraction of Thonningia sanguinea.	An HP-5MS (30 m · 0.25 mm ID · 0.25 lm) column (Agilent, USA) was used.	high purity helium	1 mL /min 1	22
04	β-Sitosterol	Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column with dimensions of 30 m × 0.25 mm i.d. and 0.25 μm film thickness.	Helium	1.3ml/min	23

05	β-Sitosterol in Michelia champaca L. Leaves and Stem Bark	2 m, 0.25 mm i.d. Zebron guard column with deactivated tubing (Phenomenex, Aschafenburg, Germany). The guard column was linked via a deactivated press ft connector (BGB Analytik, Rheinfelden, Germany) to a 30 m, 0.25 mm	helium	1.0ml/min	24

BETA SITOSTEROL ANALYSIS BY HPTLC TECHNIQUE

High-Performance Thin-Layer Chromatography (HPTLC) is a powerful technique for the analysis of β-sitosterol. It is performed on pre-coated layers using tools and is found useful for quantification. TLC is nearly universally used to teach chromatography principles. This preference is mostly due to the sample's transparency during chromatography, its ease of use, and the extremely affordable demonstration equipment. Use of multiple developments is a popular way to boost resolution under settings when capillary flow is controlled. One-dimensional or two-dimensional separations are achievable with planar chromatography.

Additionally, mobile phase velocity can be controlled externally, as in forced-flow development. Because sample chromatography is carried out in parallel, HPTLC is the fastest method of chromatography. [7]

Table 04. BETA SITOSTEROL ANALYSIS USING HPTLC TECHNIQUE

Sr No.	**Phytoconstituents**	**Stationary phase**	**Mobile phase And Detection (nm)**	**Rf and Linearity range**	**Ref no.**
01	β-Sitosterol, alpha-Amyrin, Lupeol, and n-Triacontane	Silica gel GF254 (Merck) 15 × 10 cm	Benzene: Methanol (9:1) 273nm	0.50 and 0.51 in leaves and stem-bark 200 to 1000 μg mL-1	25
02	β-Sitosterol	20 cm × 10 cm TLC aluminium precoated silica gel 60F254 plate, with 200	comprising petroleum ether: ethyl acetate: acetonitrile (8.2:	0.1–1.0 μg/band	26

		µM layer thickness	1.2: 0.1 v/v/v). 580nm		
03	Scopoletin and B-Sitosterol in Plant Extracts of Girardinia Diversifolia	a TLC A1 pre-coated silica gel plates 60 F254 (10 cm×10 cm with 0.2 m thickness, using CAMAG Lino mat V applicator.	toluene-ethyl acetate (8:2v/v) 580nm	Rf -0.91	15

BETA SITOSTEROL ANALYSIS USING HIGH-SPEED COUNTER CURRENT CHROMATOGRAPHY (HSCCC)

HSCCC is a type of counter-current chromatography that involves separating compounds based on their partitioning between two immiscible liquid phases. It removes issues between solutes and solid supports and is a crucial separation technique that has recently been applied to preparative separation and standard purification.[5]

Among the benefits of this technique, sample recovery, reduced solvent usage, effectiveness, affordability, and a large sample loading capacity are known. The UV detector and multilayer coil column were utilized by every researcher.[27]

Table 05. Beta Sitosterol Analysis Using HSCCC

Sr No.	Phytoconstituents	Instrument	Column and Revolution speed	Detector	Solvent system	Ref no.
01	beta sitosterol	Shimadzu series LC-10Avp HPLC apparatus	column (1.6 mm i.d.) volume was 234 mL. 800rpm	204nm	heptane–acetonitrile–ethyl acetate (5: 5: 1, v/v/v)0.5 mL/min.	27

Conclusion

The analytical techniques for detecting the presence of beta sitosterol have been compiled in this review. It shows variety of important biological activities thus suitable analytical techniques are required for its estimation, quantification, and separation in a variety of samples. A wide variety of analytical techniques for beta- sitosterol in different pharmaceutical formulations and biological materials are found. High-performance thin-layer chromatography, GC-MS, and high-performance liquid chromatography were found to be economical, quick, efficient, and ecologically friendly technologies.

The analysis of the compiled data indicates that High-Performance Liquid Chromatography (HPLC) is highly favoured for accurately quantifying beta sitosterol. By comprehensively assessing the methodologies employed in previous studies, analysts can enhance their understanding and optimize their own experimental approaches for analysing any drug. This review would be beneficial for analysts seeking insight into the specific combination of solvents and instruments utilized in the analysis of beta sitosterol.

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