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Bioavailability study of β-sitosterol in rat plasma after oral administration: evaluation using HPLC

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Abstract

This is an attempt to evaluate the bioavailability of β-sitosterol in rat plasma using High-performance liquid chromatography (HPLC). The developed HPLC with ultraviolet detection has been applied to predict the pharmacokinetic parameters of β-sitosterol after oral administration at a dose of 20 mg / kg bw to male albino Wistar rats (n=6), blood samples were collected at different time intervals (0, 1, 2, 4, 8, 12, 24 and 48 h). Separation was achieved on Eclipse XDB-C_{18} column (200 x 4.6 mm) using methanol: acetonitrile (30 : 70, v/v) as a mobile phase at a flow rate of 1.0 ml / min and data were analyzed using one-way analysis of variance (ANOVA). From the calibration curve, the amount of β-sitosterol was calculated. The retention time of β-sitosterol in plasma sample was 2.95. The oral administration of β-sitosterol at a dose of 20 mg/ kg bw produced significantly different pharmacokinetic parameters in Tmax, Cmax, halflife ($t_{1/2}$), area under curve (AUC) and elimination rate constant ($P<0.05$). The pharmacokinetic study of plant sterols could improve the ability to illustrate their bioavailability, understand the mechanism of drug action and help to promote the drug development phase.

Keywords: β-sitosterol, bioavailability, HPLC, plasma, AUC

Introduction

Innate traditional herbal products are increasingly used in our societies because to enhance good health and prevention of chronic diseases. Establishing the pharmacological basis and efficiency in usage of herbal medicine is a stable challenge (Wang et al., 2011). It is well known that plants are rich source of variety of compounds with nutritive and therapeutic properties. β-sitosterol, a phytoconstituents which has been conventionally used as a diuretic and has been reported in ancient medicinal history for its use in treatment of urinary problems like nephritis and prostatitis (Yadav and Srivastava, 2013). Significant improvements in symptoms and urinary flow parameters in earlier studies have also proven the efficacy of β-sitosterol in the treatment of benign prostate hypertrophy (Pagano et al., 2014). It has been reported to have various pharmacological activities such as antihypercholesterolemic, antiinflammatory, antibacterial, antifungal and antitumor properties (Kim et al., 2014; Tao et al., 2013; Hac-Wydro, 2013; Chandler et al., 1979). Thus β-sitosterol has considerable

therapeutic potential for the ailment of cancer, understanding the pharmacokinetics of β-sitosterol could help to design effective dosage for its optimal use to aid cancer chemotherapy. To this end, we examined pharmacokinetic properties of β-sitosterol in rat plasma after oral administration of β-sitosterol using simple, sensitive, precise, rapid, accurate, reproducible, cost-effective and suitable HPLC method along with UV detector.

Materials and methods

Chemicals

All the chemicals used in the experiments were of HPLC grade. β-sitosterol was procured from Sigma Aldrich Chemical Company, (St. Louis, MO, USA) and the solvents were procured from Hi-media Laboratories Pvt, Ltd., Mumbai, India.

Chromatographic System (HPLC-with UV detector)

Chromatographic separation was performed with high performance liquid chromatography (Pharma Spec. UV-1700, Shimadzu, Kyoto, Japan) connected to a Hewlett Packard (Palo Alto, CA) photodiode array UV–visible detector. UV detector was set at 210 nm. β-sitosterol extracted from plasma samples were analyzed on a Eclipse XDB-C18 (Shimadzu) 200×4.6 mm i.d. column (sample amount, 20 µl). The stationary phase of the column was a Diamonsil C18 (5-mm particle size). The mobile phase containing methanol: acetonitrile (30:70, v/v) delivered at a flow rate of 1.0 ml/min. The experiments were conducted at 30° C. Mobile phase and samples were filtered through a 0.45 µm membrane filter (Millipore) and degassed by sonication. HSM-LACHROM Multi HSM manager chromatographic software was used for data acquisition.

Preparation of Standard Solution and Calibration

The stock solution of β-sitosterol (1 mg mL^{-1}) was prepared in methanol. The stock solution was quantitatively transferred to give a solution of appropriate concentration range of β-sitosterol (2 – 10 µg mL^{-1}). Standard solutions were prepared by dilution of the stock solution. These solutions were spiked into drug – free rat plasma samples to determine the recovery, precision, accuracy, and detection limit of the HPLC method.

Animal Study

The developed HPLC method was used in a pharmacokinetic disposition study after oral administration of β-sitosterol (20 mg/kg body weight) to male Wistar rats (6-7 weeks old, 120-180g). Venous blood samples were collected at 0, 1, 2, 4, 8, 12, 24, 48 hrs post dose and collected in heparinised tubes. Blood samples were immediately centrifuged at 3000 rpm for 5 min and harvested plasma samples stored at -20°C until analysis. The local institutional animal ethics committee (Registration number 160/1999/CPCSEA) of Annamalai University approved the experimental design (Proposal No. 1041: dated 06.08.2013). The animals were maintained following the principles and the guidelines of the ethical committee for animal care of Annamalai University in accordance with the Indian National Law on animal care and use.

Sample Preparation

Plasma samples (0.1 ml) were shaken with 1.0 ml methanol for 2 min and centrifuged at 3000 rpm for 10 min. The methanol extract was transferred to dry tube. The procedure was repeated twice and the methanol extracts collected were dried at 40ºC under a nitrogen stream. The residue was dissolved in 100µl methanol and then injected into the chromatographic system.

Method Validation

System suitability tests were used to ensure reproducibility of the equipment. The test

was carried out by injecting 20 µl of mixture of standard solution at assay concentration of β-sitosterol. The experiment was performed three times and the mean was used for the calculations. The data was analyzed by linear regression.

Calculation

In order to quantitate the samples, an external standard method was employed. Standards of known concentrations of β-sitosterol were used as an external standard. The concentration of the standard solution was measured using a spectrophotometer (Pharma Spec. UV-1700, Shimadzu, Kyoto, Japan), and the employed extinction coefficient E1cm, 1% at 210 nm in light petroleum. The peak area of β-sitosterol was determined, and the pharmacokinetic parameters were calculated from the resulting means and spectrophotometrically measured β-sitosterol concentration.

Statistical analysis

The values obtained for β-sitosterol content is an average of at least triplicate determinations. Comparison of means of the measurements, using a significant level of $P < 0.05$ was performed by one-way analysis of variance.

Results

The developed HPLC method was used for simultaneous determination of β-sitosterol from plasma sample. The peak area of β-sitosterol was measured when sample working solution was injected. From the calibration curve, the amount of β-sitosterol was calculated. Figure 1-7 shows HPLC Chromatogram of β-sitosterol standard and plasma from rat at different time intervals (1, 2, 4, 8, 12, 24) after oral administration of β-sitosterol at dose of 20 mg / kg bw. Retention time of β-sitosterol was detected at 2.95 and maximum concentration was reached after 2h in rat plasma after oral administration of β-sitosterol (20 mg / kg bw) and not detectable in plasma after 48h. The wave length of 210nm was used as the detection wave length at which β-sitosterol shows the greatest absorption. Absorption and elimination of β-sitosterol is followed using changes in the concentration of β-sitosterol in the HPLC profile. Figure 8 shows chromatogram of plasma concentration-time profiles of β-sitosterol taken at 1, 2, 4, 8, 12 and 24 hours after the oral administration of β-sitosterol. The various concentration of β-sitosterol in plasma of rat generated the pharmacokinetic parameters after oral administration using the absorption-elimination curve was tabulated [Table 1].

Table 1: Pharmacokinetic parameters of β-sitosterol (20 mg / kg bw) in male Wistar rats after oral administration (n=6).

PK Parameters	Mean ± SD
C_{max}	43.07 ± 2.11
T_{max}	2h ± 0.1
AUC	182.67 ± 8.69
$t_{1/2}$	2.94h ± 0.14
K_{el}	0.236 ± 0.011

C_{max} = Maximum plasma concentration, t_{max} = Time for maximum plasma concentration, AUC= Area under plasma concentration time curve, $t_{1/2}$ = Elimination half life, K_{el} = Elimination rate constant. Values that are not sharing common superscript in the same column differ significantly at $P < 0.05$ was performed by one-way analysis of variance.

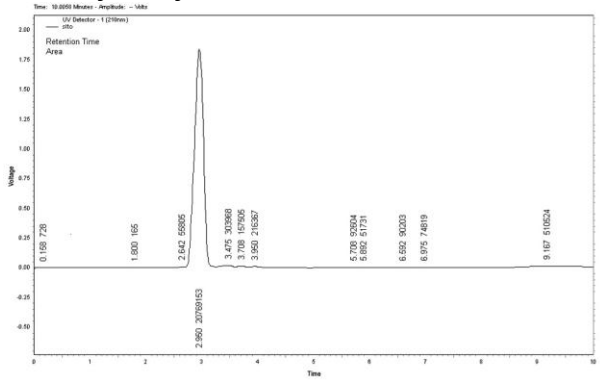

Fig. 1: HPLC Chromatogram of β-sitosterol standard.

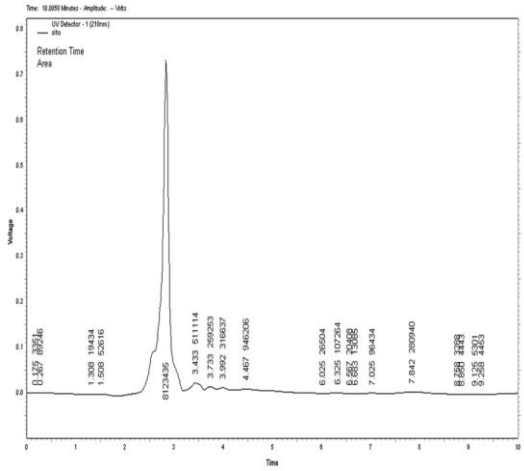

Fig. 2: HPLC Chromatogram of plasma sample 1h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

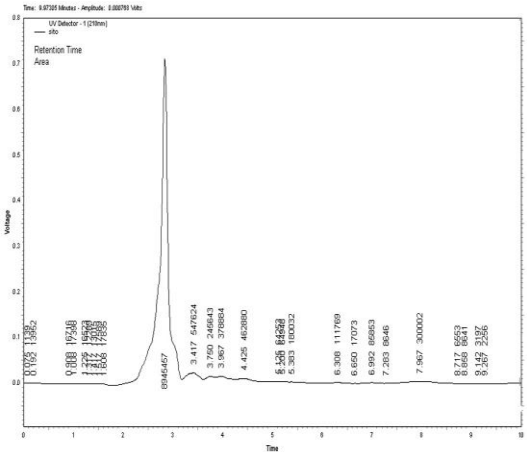

Fig. 3: HPLC Chromatogram of plasma sample 2h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

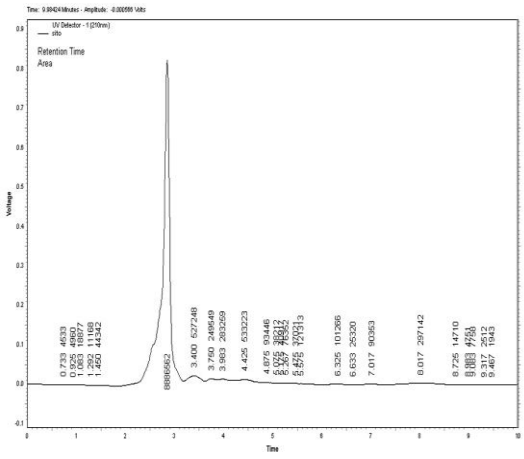

Fig. 4: HPLC Chromatogram of plasma sample 4h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

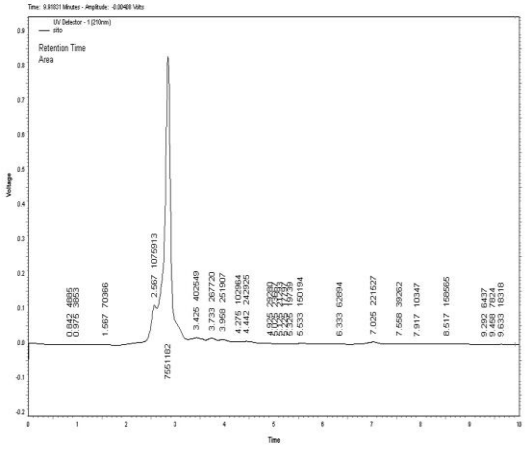

Fig. 5: HPLC Chromatogram of plasma sample 8h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

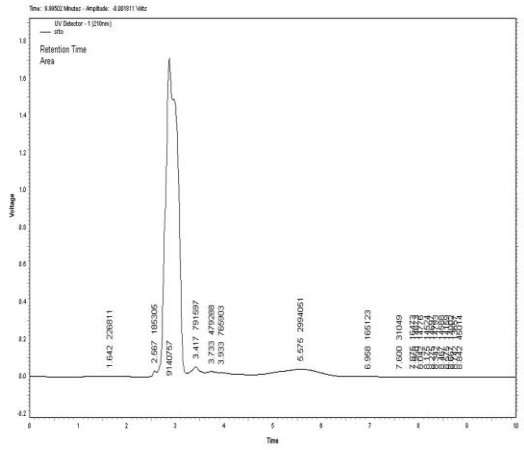

Fig. 6: HPLC Chromatogram of plasma sample 12h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

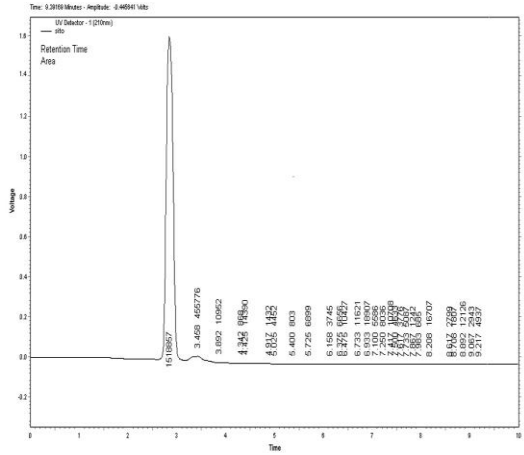

Fig. 7: HPLC Chromatogram of plasma sample 24h after an oral administration of β-sitosterol at dose of 20 mg / kg bw (n=6).

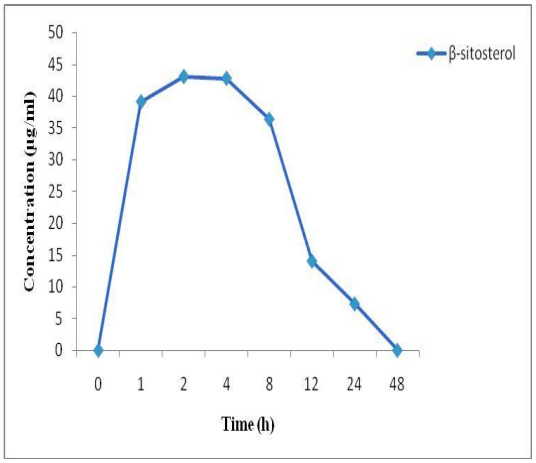

Fig. 8: Plasma concentration-time profiles of β-sitosterol after oral administration in rats at dose of 20 mg / kg bw (n=6).

Discussion

β-sitosterol is a natural micro-nutrient which is widely found in *Nigella sativa*, *Serenoa repens*, *Pygeum africanum*, sea-buckthorn, wolfberries, *Mirabilis jalapa* (Siddiqui et al., 1990), *Cannabis sativa*, *Urtica dioica* (Kopyt'Ko et al., 2012) and *Wrightia tinctoria* (Srivastava, 2014). Plant sterols are relatively inexpensive and can safely be used in conjunction with conventional therapies in management of human health. Of particular interest is the question of bioavailability to assess to what degree and how fast compounds are absorbed after administration of an herbal medicine (Bhattaram et al., 2002). The therapeutic efficacy and safety of many drugs commonly used in clinical practice can be augmented by individualization of their dosage (Patel, 2013). Knowledge of absorption, distribution, metabolism and excretion for most herbal preparations is important to establish markers which can be used to monitor the absorption-elimination process. Use of natural products has been proven to be a safe and effective nutritional supplement and has shown amazing potential benefits in many diverse applications (Torwane et al., 2014).

However, still more scientific evaluation and clinical trials are required to establish its therapeutic efficacy. Understanding the disposition of phytosterols at the gastro-intestinal (GI) tract and systemic level is important as they are used as functional food ingredients and this builds further on our mechanistic understanding (Wasan et al., 2001). The primary molecular mechanism is believed to be physico-chemical competition between cholesterol and phytosterols for micellar incorporation. The source of β-sitosterol in the body is known to be dietary as it is not synthesised in humans. Therefore, it will be of great therapeutic interest to evaluate the relative bioavailability of β-sitosterol. To gain a better insight into the exact bioavailability and the disposition characteristics of xenosterols, using the main β-sitosterol as an example, we screened through this pharmacokinetic study.

Conclusion

The oral administration of β-sitosterol at a dose of 20mg/kg bw produced significantly effective pharmacokinetic parameters in T_{max}, C_{max}, half life, AUC and elimination rate constant ($p< 0.05$). This kind of pharmacokinetic studies of active compounds could improve the ability to illustrate their mechanisms of action and help to promote the drug development phase in to lead development.

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