A newly modified HPLC method for estimation of dutasteride in prepared niosomes

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Abstract:

A modify, fast, accurate and precise HPLC conditions were developed and validated for the determination of dutasteride (DT) in niosomes derived from proniosomal gel. The work has shown enough isolation for DT from other contents of the niosomes. The isolation of DT was achieved on C18 column (3µm, 125 × 4.6 mm). The mobile phase was a mixture of acetonitrile: water in a ratio of (50:50) and the flow rate was 1.5 mL per minute. The wave length used to detect dutasteride was 210 nm and the ejection volume was 100 µL. The calibration curve of concentrations range from 50 to 0.3 µg/mL obeys Beer-Lambert's law (R^2 = 1) for DT. The limits of detection were 0.3 µg/mL for DT with high accuracy and precision which approved that no interaction between the drug and other niosomal content unlike the ordinary UV-spectroscopy and the reported HPLC procedure for DT.

Keywords: Acetonitrile, Dutasteride, Modified HPLC condition, Niosomes.

طريقة تحليل معدلة لفحص الدوتاستيرايد في مستحضرات النايوسوم باستخدام تقنية الكروملتوغرافي السائلة ذو الضغط العالي

الخلاصة:

تم تطوير طريقة سريعة، دقيقة وفعالة باستخدام تقنية الكروملتوغرافي السائلة ذو الضغط العالي لتحديد الدوتاستيريد في النايوسوم المستمدة من هلام البرونايوسوم. وقد أظهر العمل ما يكفي من العزلة لدوتاستيرايد من محتويات النايوسوم الأخرى. تم التوصل إلى عزل لدوتاستيرايد على عامود 3 (C18 مايكروميتر، 125 × 4.6 مم). المحلول الناقل هو خليط من الأسيتونتريل: الماء في نسبة (50:50) ومعدل التدفق هو 1.5 مل في الدقيقة الواحدة. طول الموجة المستخدمة للكشف عن الدوتاستيريد هو 210 نانومتر وحجم العينة 100 ميكرولتر. يتراوح منحنى المعايرة من تركيزات من 50 إلى 0.3 ميكروغرام / مل وينطبق على قانون بير لامبرت(R^2 = 1) . وكانت حدود الكشف هي 0.3 ميكروغرام / مل لدوتاستيريد. هذه الظروف تسهم في التغلب على كل التداخلات التي رافقت الكشف بالطيف الأشعة فوق البنفسجية العادية وظروف سائل فاصل للون عالي الكفاءة لدوتاستيريد في نايوسوم المشتقة من هلام البرونيوسوم.

Introduction:

Dutasteride is an inhibitor of 5 alpha-reductase. DT chemical name is (1S,3aS, 3bS,5aR,9aR,11aS)N[2,5bis(Trifluoromethyl) phenyl]-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a, 6,9b, 10 ,11 dodecahydroindeno [5,4-f] quinoline -1-carboxamide. DT powder has a white to pale yellow color (1). The chemical structure is shown in figure 1.

5-alpha-reductase is an intracellular enzyme that coverts testosterone to 5-alpha-dihydrotestosterone (DHT). DHT is responsible for prostate gland enlargement and initiate and progress the benign prostate hypertrophy. Inhibition of 5-alpha-reductase reduces the DHT level significantly, which contributes to the treatment of benign prostate hypertrophy (2).
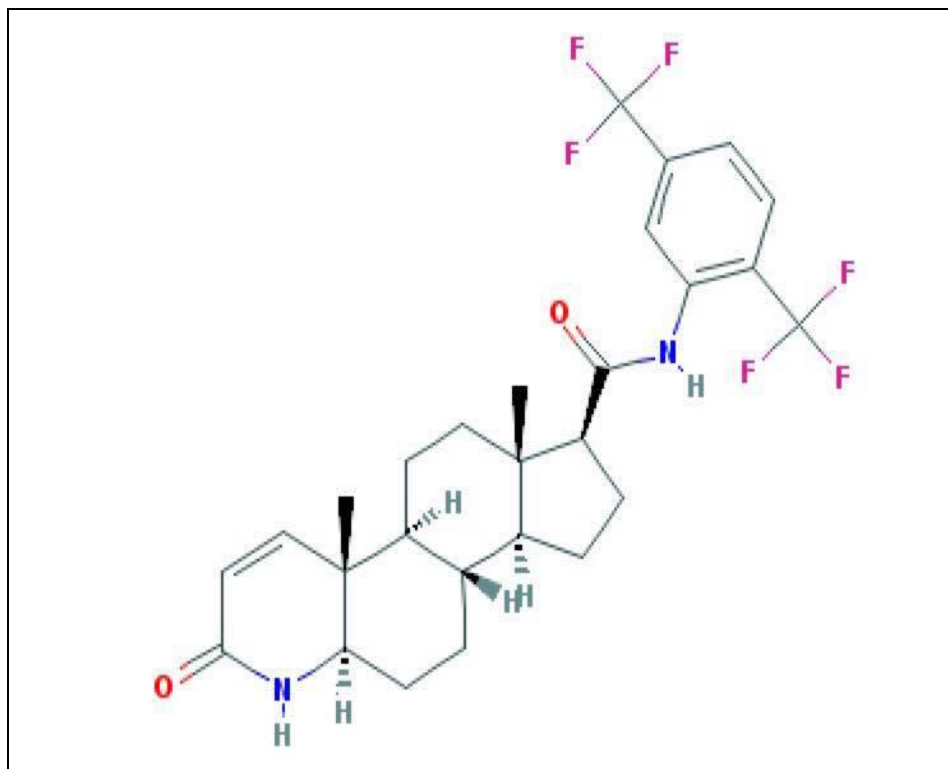
Figure-1: The structure of DT

Very low water solubility of DT (0.9 µg/ml) makes it marketed as a soft gelatin capsule in which DT is dissolved in an organic solvent [3]. However, soft gelatin capsules have some problems such as high cost, limited margin of excipients used and the need for specialized equipments[4]. Therefore, many techniques have been developed to improve solubility and dissolution of DT to improve the bioavailability of the drug including niosomes which usually contain cholesterol and surfactants.

The DT has two important λmax 240 nm and 210 nm which can be used to estimate the concentration of the drug in in-vitro and in-vivo studies using UV-spectrum. The more practical λmax to be chosen is 240nm since it has acceptable intensity and low interferance. This λmax cannot be used for DT determination in niosomes since, cholesterol (which is one of the niosomal components) has the same λmax. the interaction will lead to inaccurate results, especially, DT has low dose (0.5 mg).

The more advanced method for DT determination is using high performance liquid chromatography (HPLC). The traditional HPLC conditions used for DT concentration determination was C18(2) column (5 µm, 250 × 4.6 mm) and the mobile phase consisted of a mixture of acetonitrile: water (60:40 v/v) and a flow rate of 1.0 mL per min. The injection volume was 20 µL, and detection was performed at 210 nm. The previous method used to detect DT in solid dispersion (5), inclusion method (6) and solid- supersaturatable self-microemulsifying drug delivery system (7), but cannot be applied for niosomes ,since,it shows peak interference between the drug and other niosomal content.

The objective of this work is to create new HPLC conditions that resolve the interaction problems of DT in the prepared niosomal formula.

Materials and methods:
Materials:

DT powder was purphased from Hyperchem, Co (China). Cholesterol from BDH Co. (UK). Acetonitrile- HPLC grade and methanol were obtained from Biosolve

B V (France). Water used is purified de-ionized distilled water.

Methodology:

DT characterization:

Melting point determination:

Melting point usually determined using capillary method that is described in the united state pharmacopeia (USP). The capillary tube was sealed from one side and the DT powder was filled from the other side and the drug powder melting temperature was recorded [8].

Estimation of UV Absorption Maxima (λmax):

A stock solution of DT was prepared by dissolving 10 mg of the API in 100 mL methanol to get a concentration of 100 µg/mL. The standard solution was diluted to get a concentration 50 µg/mL. The later concentration (50 µg /mL) was scanned in the UV-visible spectrophotometer at the range of 200-400 nm, and the λmax was recorded.

Characterization of DT by HPLC (specificity):

The prepared standard solution of DT (50 µg/mL) was injected in the HPLC to determine the retention time of DT. A modified HPLC condition was used for DT prediction. The ejection volume was100 µL and the column was C18 column (3µm, 125×4.6 mm). The mobile phase was a mixture of acetonitrile: water in a ratio of (50:50) and the flow rate was 1.5 mL per minute. The wave length used to detect DT was 210 nm. This method was applied to estimate DT in standard solution and in niosomal vesicles as well as its saturated solubility.

Linearity:

Calibration curve of DT was constructed by preparing a series of standard solutions ranging from 50 µg/mL to 0.3 µg/mL. These standard solutions were prepared by adding a specific volume from stock solution into 25 mL volumetric flask and complete the volume to 25 mL with methanol. The concentrations of the of the

prepared diluents were 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.4, 0.3 µg/mL. The absorbance of the standard solution was obtained at 210 nm using HPLC conditions mentioned above. The area under the peak (AUP) for each standard solution is recorded.

Assay of Accuracy and precision:

The accuracy and precision were assessed by taking 3 different concentrations of standard solutions namely (50, 5, 0.5 µg/mL) and measuring the AUP of each concentration in three successive days. The (ANOVA) method was used for estimation the variability in samples of the same concentration analyzed in the three successive days [9].

Robustness:

The method identify the effect of the changes in the HPLC parameters, such as mobile phase composition, temperature, flow rate, etc. on the separation. Robustness testing was performed in the temperature range from 25 °C to 40 °C and altered flow rate of 1 mL/min to 1.5 ml /min [9].

Determination of the saturated solubility of DT:

The saturated solubility of DT was determined using different media such as water and 0.1 N HCl containing 2% sodium dodecyl sulfate (SDS). The saturated solubility is performed by adding an excess quantity of drug into a 50 mL volumetric flask containing 25 mL from the specific medium. The volumetric flask placed in magnetic stirrer at 25 ^{0}C for 24 hours and then sonicated   for 10 min. This procedure provides a sufficient time and stress conditions to produce the saturated solubility [10]. The saturated concentration is measured using the developed UV detector at λmax 210 nm. The area under the peak (AUP) for each solution was recorded and the concentration was estimated using the constructed calibration curve.

Entrapment efficiency of DT in niosomes derived from proniosomal gel:

The proniosome gel was prepared by simple coacervation method and the niosomes were derived from the proniosomal gel be adding 10 mL of preheated phosphate buffer at pH 7.4. The formula contains DT (0.5 mg), cholesterol (24.9 mg), Tween 20 (36.7 mg) and span 60 (13.8mg). The niosomal dispersion was placed in a centrifuge and run at 14000 rpm. The niosomal vesicles precipitated and unloaded DT was found in the supernatant layer [11]. The concentration of DT in the supernatant layer estimated by the developed HPLC. The entrappment efficiency was calculated using the following equation:

Entrappment efficiency (%) = [(dt - df)/ dt] × 100

Where: dt is the total concentration of DT and df is the concentration of free DT.

Results and discussion:

Melting point determination:

The observed melting point of DT was 246 °C which is agreed with reported data indicating the purity of the drug [1].

Estimation of UV Absorption Maxima (λmax):

Scanning the standard DT solution (50 µg/mL) revealed that DT has two important λmax 240 nm and 210nm (as shown in figure 2) which are consistent with the reported value [12,13].
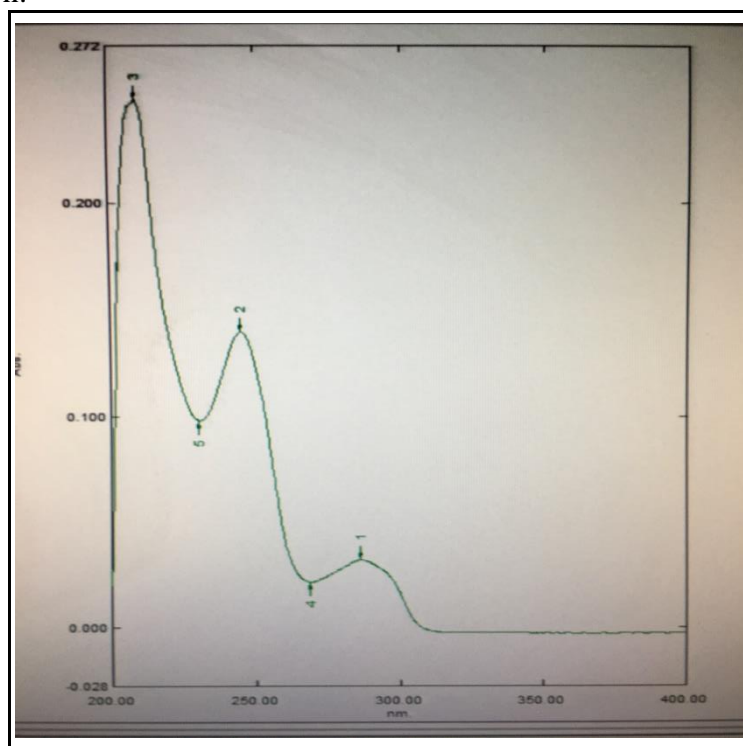

Figure-2: The UV-spectrum of DT in methanol.

Characterization of DT by HPLC (specificity):

A modified HPLC condition were used in which the mobile phase is acetonitrile: water in a ratio of (50:50) and the flow rate is 1.5 mL per minute at 210 nm. This condition gave an intense, sharp DT peak at 13.58 min in both standard solution (in methanol) and in niosomal preparation as shown in figure-3.
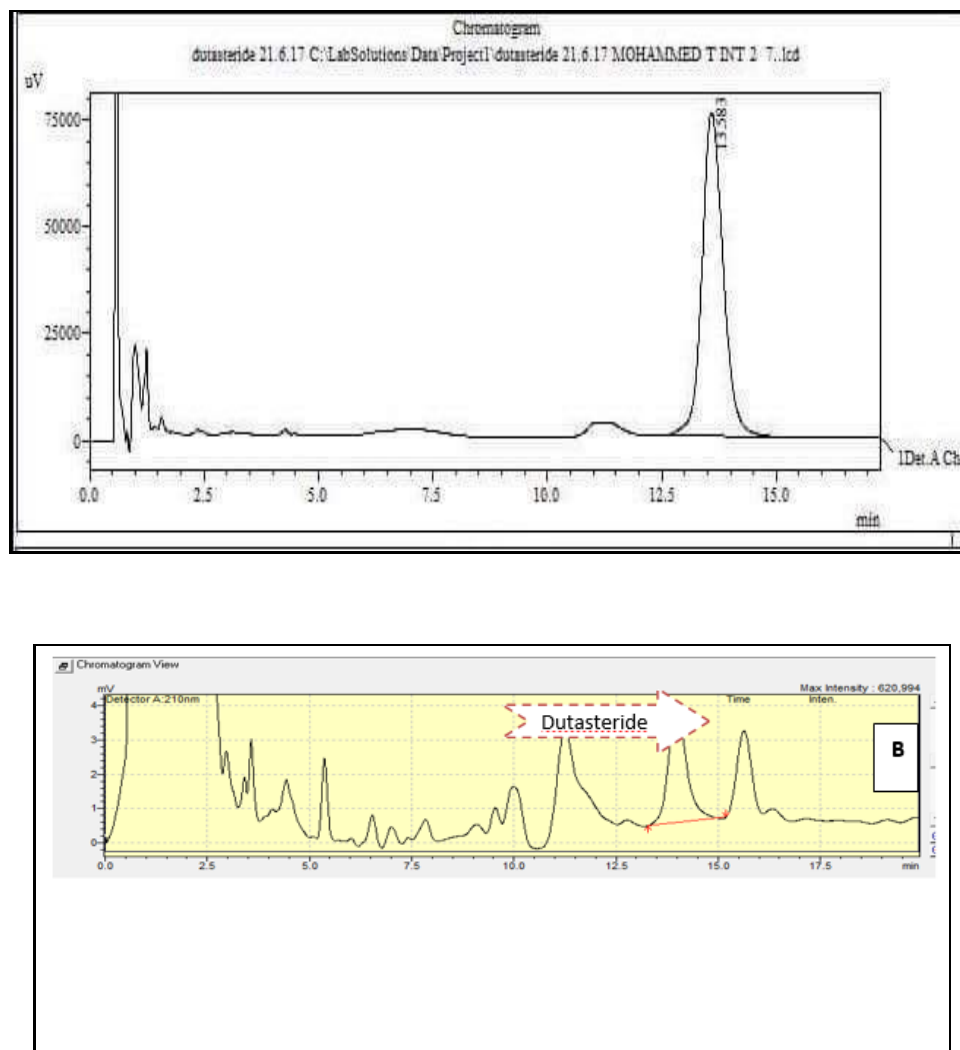
Figure3-: HPLC spectrum of DT (A) in methanol (B) with niosomal components.

Linearity:

Figure 4 reveals the calibration curve of DT obtained from plotting the concentrations of standard solution versus AUP at 210 nm. The straight line obtained indicated that the calibration curve obeys Beer-Lambert's law within the concentration of standard solutions used
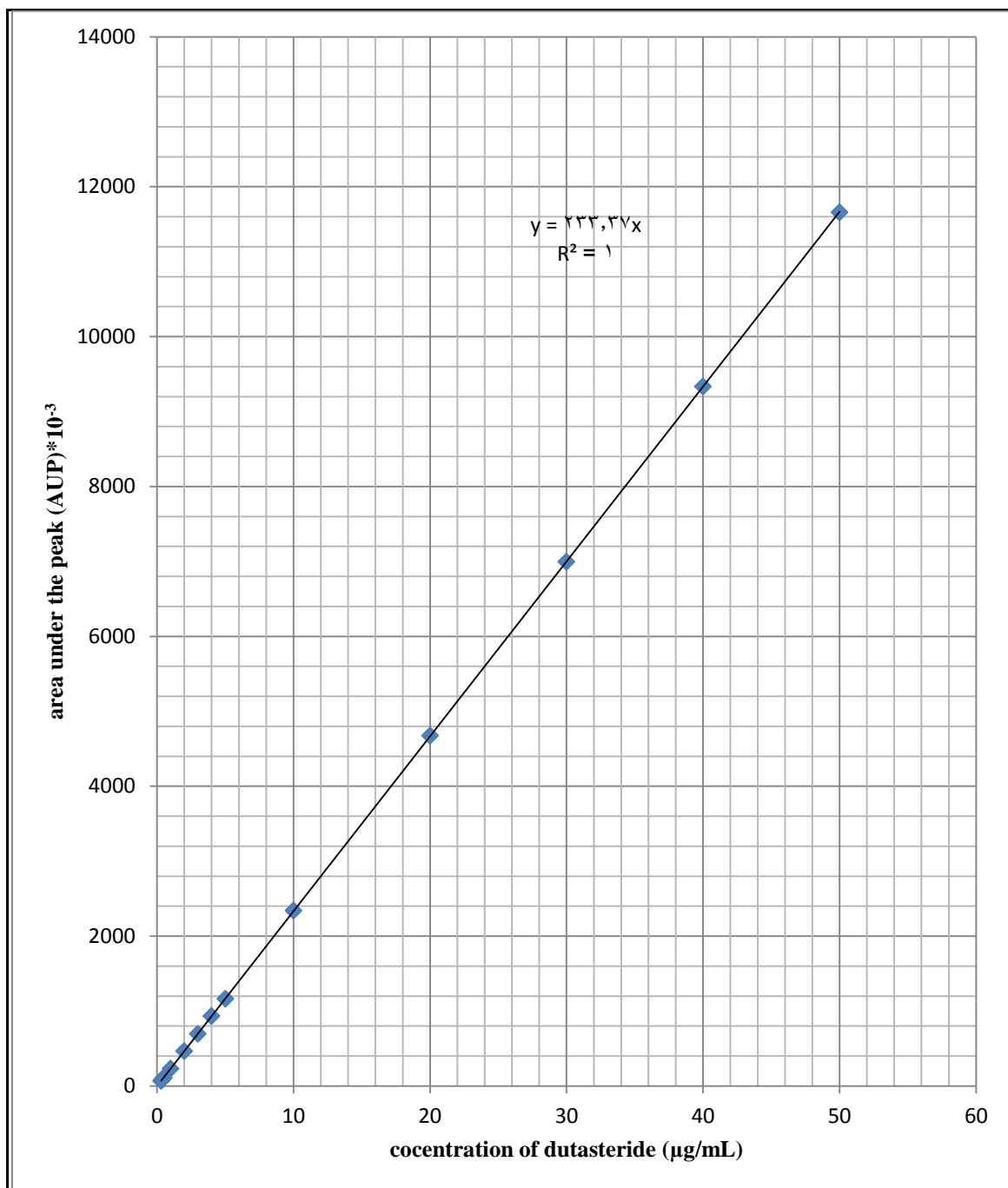
Figure-4: Calibration curve of DT in methanol, using HPLC.

Assay of accuracy and precision:

Table 1 shows the percentage of accuracy for the recovered analyte using the assay method with acceptable error level less than ±2%[14].

Table-1: Accuracy data of standard solutions

Theoretical concentration (µm/ml)	Mean Recovered concentration	Recovery (%)
50	49.5± 0.54	99
5	4.9±0.06	98
0.5	0.509± 0.01	101.8

Robustness:

Upon changing the temperature (25 to 45 ᵒC) and changing the flow rate from 1 to 1.5 mL/min, the change in retention time was less than 2% from the original value (13.58) which is within the acceptable limit [15].

Determination of the saturated solubility of DT:

The newly developed HPLC method was applied to estimate DT saturated solubility in water and 0.1 N HCL containing 2 % SDS. The saturated solubility of dutaseride in water was 1.03 µg/mL which is agreed with the reference data [3]. The other medium (0.1 N HCL containing 2 % SDS) is FDA official dissolution medium of oral DT and the saturated solubility was found to be 30 µg/mL.

Entrapment efficiency of DT in niosomes derived from proniosomal gel:

The amount of drug in the niosomal vesicles was estimated by the developed HPLC where a sharp, characteristic peak at 13.58 min (retention time) well separated from other peaks of the niosomal content was observed.

The entrapment efficiency was calculated and it was 98% indicating the sensitivity, accuracy and precision of the developed HPLC for the estimation of DT in niosomal preparation.

Conclusion:

The new HPLC conditions have acceptable specificity, linearity, accuracy, precision, robustness. Also, these HPLC conditions gives an intense sharp peak for DT after assaying the entrapment efficiency without interfering with peaks of other niosomal components.

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