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Original Research Article

Determination of dehydroepiandrosterone in dietary supplements by reversed-phase HPLC

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Abstract

Purpose: *To develop a reversed phase high performance liquid chromatography (HPLC) method for the determination of dehydroepiandrosterone (DHEA) in dietary supplements.*
Methods: *A reversed-phase high performance liquid chromatography (HPLC) method was developed for the determination of DHEA in dietary supplements. An isocratic system consisting of methanol and water (70:30 v/v) was run at a flow rate of 1 mL/min on a C18 HPLC column to achieve the separation. The method was validated with regard to linearity, intra-day and inter-day precision, and limits of both detection and quantification.*
Results: *The method achieved a retention time of 10.8 min, a resolution of 4.12, a detection limit (LOD) of 50 ng/µL, a quantification limit (LOQ) of 166.7 ng/µL and a label claim of 108.6 % with a relative standard deviation (RSD) of 0.38 % over a range of 0.0625 – 0.50 mg/mL with a correlation coefficient of 0.9997.*
Conclusion: *The method is simple, cost effective, time-saving and reliable for determining DHEA when compared to other reported methods in literature. Thus, it will be of benefit to manufacturers of this dietary supplement to adopt the method for quantitative laboratory analysis.*

Keywords: *Dehydroepiandrosterone, Prasterone, Dietary supplement, HPLC, Method development, Validation*

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INTRODUCTION

Dehydroepiandrosterone (DHEA; Figure 1), also known as Prasterone, is a major endogenous steroid hormone that functions mainly as a metabolic intermediate in the biosynthesis of androgenic and estrogenic sex steroids [1]. The production of DHEA is highly affected by age as it reaches its peak between the ages of 20 and 30. After that, it steadily declines and by the ages of 70 to 80, DHEA levels drops to about 10 - 20 % of what it usually is in a young adult [2].

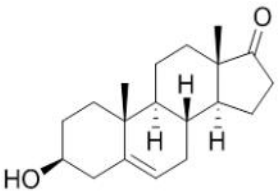

Figure 1: The structure of dehydroepiandrosterone (DHEA)

In 1934, DHEA was isolated, for the first time, from human urine. After the passage of the Dietary Supplement and Health Education Act (DSHEA) of 1994, the availability of DHEA as a naturally occurring dietary supplement has increased [1]. DHEA supplementation energizes athletes and people that perform regular physical activities, improves memory, enhances concentration and attention, activates the immune system and increases testosterone levels [3]. DHEA is available commercially in the form of tablets and capsules, with a dosage that ranges from 5 to 100 mg of DHEA, with 25 and 50 mg being the most common doses. The available formulae of DHEA could include it as a sole active ingredient, or it could be combined with other steroidal or vitamin supplements. Moreover, DHEA could be combined with some herbal extracts, such as gingko, ginseng and yohimbine [1].

The detection of DHEA and its metabolites in biological fluids is usually carried out using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) [4-7]. However, for determining DHEA in food additives, high performance liquid chromatography (HPLC) is frequently utilized [8,9]. However, most of the HPLC methods that are currently applied rely on normal stationary phases or buffer solutions as mobile phases and as such, they are expensive, non-versatile and complicated [10,11]. This work implements a reversed phase-HPLC method for the assay of DHEA in dietary supplements, aiming to save time, cost, and effort in the analysis.

EXPERIMENTAL

Samples and reagents

Dehydroepiandrosterone standard was obtained from Sigma (St Louis, MO, USA). The dietary supplement, 10 bottles of DHEA 50 mg tablets from three different batches were obtained from American Health and Wellness Pharma (AHW) (NC, USA). Deionized Water (LabChem®, USA) and HPLC-grade methanol (fulltime®, China) were used as solvents for the mobile phase.

Instrumentation

The HPLC instrument DIONEX UltiMate™ 3000 was obtained from Thermo Fisher Scientific, Waltham, MA, USA and is composed of an ultraviolet–visible detector, a pump, an autosampler and uses the Chromeleon™ 6.0 software. The ultrasonic water bath was purchased from JeioTech®, Korea.

Chromatographic conditions

An isocratic reversed phase HPLC separation was carried out using a mobile phase of methanol and water (70:30 v/v) and a C18 column (Eurospher-100, 150 mm × 4.6 mm, 5µm) with a flow rate of 1 mL/min. The elution system was set at a wavelength of 210 nm and with an injection volume of 20 µL.

Test solutions

Preparation of the standard solutions of DHEA

A 0.5 mg/mL stock solution of DHEA was prepared by dissolving DHEA in the prepared mobile phase (methanol:water 70:30 v/v) followed by sonication for 5 minutes. Then, a series of DHEA solutions with concentrations of 0.5, 0.25, 0.125, and 0.0625 mg/mL were prepared by diluting the stock solution with the same solvent. Thereafter, the resulting solutions were filtered through a membrane filter with a pore size of 0.45 µm before being injected into the HPLC instrument.

Preparation of DHEA test solution

Five tablets, each containing a 50 mg of DHEA, were weighed before being crushed by a mechanical grinder with the average weight of one tablet being 300.69 mg. After that, 28.7 mg of the crushed tablet mass was weighted and added to 20 mL of the mobile phase (solvent), followed by 10 minutes of sonication in an ultrasonic bath with intermittent stirring and the subsequent solution was then diluted up to 50 mL in a volumetric flask [3]. Finally, the resultant solutions were filtered through a membrane filter with pore size of 0.45 µm and injected into the HPLC instrument.

Method validation

Linearity

Stock solutions of DHEA with concentrations of 0.5, 0.25, 0.125, and 0.0625 mg/mL were prepared as mentioned in Preparation of the

standard solutions of DHEA. The data of peak area versus the concentration of DHEA were then subjected to linear regression analysis using Microsoft ® Excel®.

Precision

Intra-day and inter-day precision values were determined by analyzing samples five times a day on five separate days. Precision was expressed as a relative standard deviation (RSD) values.

Detection and quantification limits

The mobile phase was injected six times and the noise level was determined to estimate the limits of both detection and quantification. The limit of detection was considered three times the noise value, whilst the limit of quantification was considered ten times the noise value.

RESULTS

Method optimization

Various conditions were optimized during the method development to achieve appropriate, fast, and accurate method for the determination of DHEA in dietary supplements. Different mobile phases were tested, all composed of different ratios of methanol and water and detection was carried out at different wavelengths. Mobile phase composed of methanol:water (70:30 v/v) at a flow rate of 1 mL/min at 210 nm generated a sharp peak at a reasonable retention time (*ca*. 10.8 min) in both the standard DHEA solution and the tested DHEA dietary supplement solution (Figure 2 and Figure 3).

Validation

Linearity was determined for the used chromatographic system at a concentration range of 0.0625 – 0.5 mg/mL. The peak area was plotted against the concentration to generate a calibration curve, where the absolute coefficient of correlation was 0.9997 and a linear relationship was noticed. In addition, the regression equation was: y = 294.8 x.

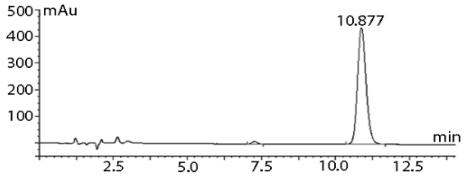

Figure 2: Chromatogram of a standard 0.5 mg/mL solution of DHEA (t_R = 10.877 min)

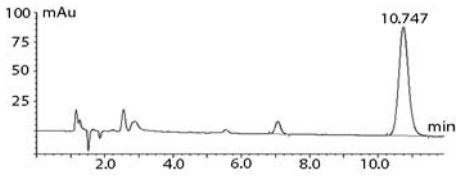

Figure 3: Chromatogram of a DHEA dietary supplement solution (t_R = 10.747 min), with a concentration of 0.574 mg/mL of the crushed tablet mass that corresponded to 0.106 mg/mL of DHEA after using the created calibration curve to calculate the concentration of DHEA in the solution of the tablet mass

The system suitability parameters are presented in Table 1. The table included both the intraday and the interday precisions, both expressed as repeatability of peak areas and retention time. Moreover, both the tailing factor and number of theoretical plates were used as tools to assess the precision of the proposed method. As the table shows, all the mentioned values exhibited good precision as revealed by their relative standard deviation (RSD) values, indicating the proposed method to be precise. Furthermore, both detection and quantification limits (LOD and LOQ) were calculated as 50 ng/mL and 166.7 ng/mL, respectively. In addition, the proposed method demonstrated good resolution as indicated by the values presented in Table 1.

Table 1: System suitability parameters

Parameter		DHEA standard (0.5 mg/mL)	DHEA as a dietary supplement (0.106 mg/mL)
Intra-day	Area under the peak	143.93, RSD=1.73%	29.13, RSD=2.93%
	t_R (min)	10.93, RSD=0.76%	10.82, RSD=0.84%
Inter-day	Area under the peak	142.75, RSD=1.57%	28.07, RSD=4.27%
	t_R (min)	10.96, RSD=1.52%	10.93, RSD=1.73%
Tailing factor		1.16, RSD=0.99%	1.10, RSD=0.91%
Number of theoretical plates		6738.6, RSD=3.39%	7220.3, RSD=3.48%
LOD (ng/mL)		50	50
LOQ (ng/mL)		166.7	166.7
Resolution		5.26	4.12
t'_R (min)		7.37	7.60

Values are presented as means. repeatability (n = 5). DHEA = dehydroepiandrosterone, t_R = retention time, t'_R = corrected retention time, RSD = relative standard deviation, LOD = detection limit, LOQ = quantification limit

Label claim

The constructed calibration curve was employed to calculate the actual amount of DHEA that a tablet contains. After that, label claim (L) was calculated by dividing the actual weight of DHEA by the labeled DHEA weight, using the following equation:

L (%) = (Wa/Wl)100

where Wa and Wl are the actual and labelled weights of DHEA, respectively.

Repeatability and precision (as designated by RSD values) for DHEA label claim are shown in Table 2.

Table 2: Label claim of DHEA in dietary supplement tablets

Labeled DHEA content	Calculated DHEA content	Label claim (%)
50 mg	54.1 mg	108.1
50 mg	54.4 mg	108.7
50 mg	54.5 mg	108.9
	Mean=54.3	Mean=108.6
	RSD=0.38 %	RSD=0.38 %

DHEA = dehydroepiandrosterone, RSD = relative standard deviation

DISCUSSION

The value of this work originated from its simplicity and the low cost of the presented method- implementing a C18 stationary phase with an isocratic mobile phase and a flow rate of 1 mL/min coupled to a UV detector to obtain an analyte peak at 10.8 min. As mentioned earlier, a lot of the currently employed methods used to determine DHEA in food supplements rely on normal stationary phases that are of higher costs compared to reversed stationary phases and limited selection of solvents. On the other hand, others use buffers in mobile phases which includes additional lab work, costs and risk factors to the separation process which are all avoided in our proposed method. For example, Ivanova *et al* used a diol stationary phase in the separation process with the aid of an isocratic flow of acetonitrile:water (98:2 v/v) at 1 mL/min, but the peak of DHEA was only obtained after 31 min of elution [3].

Meanwhile, Aboul-Enein *et al* used a C18 reversed phase HPLC column and eluted DHEA after 6.8 min at a flow rate of 1 mL/min. However, the implemented mobile phase had to be adjusted to pH 5 using 5 g/L sodium acetate buffer solution [12]. Moreover, Thompson *et al* implemented reversed phase HPLC to achieve the separation of DHEA in dietary supplement products, but their method relied on the use of phosphate buffer solution to adjust the pH to 3.50 [1]. In addition, Rush *et al* used a reverse-phase HPLC system with acetonitrile:water (45:55 v/v) as a mobile phase with detection was carried out at 207 nm. The DHEA was eluted at 8.0 min. However, acetonitrile is more costly than methanol [13]. Nonetheless, the present work utilized an isocratic, a C18 reversed phase column and a mobile phase composed of methanol and water. Thus, making this method of analyzing DHEA simple, cost efficient and could be carried out with no major limitations in labs that analyzes nutraceuticals, where the simplest method of analysis is always sought after.

CONCLUSION

Interest in dietary supplements has been growing over the decades, hence, robust analytical techniques are needed as quality control tools for these products. DHEA is no exception as it is increasingly used to slow signs of aging, improve physical performance and to increase sex hormone levels. A method for determining DHEA concentration in dietary supplements using a reversed HPLC stationary phase is here proposed as a quality control tool. The developed method is simple, cost-effective, reproducible, and ensures rapid and successful separation of DHEA from the accompanying excipients.

DECLARATIONS

Acknowledgement

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Conflict of interest

There is no conflict of interest with regard to this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yahia Z Tabaza and Kamal M Mansi analyzed the results and took part in writing and revising the manuscript. Hanan A Azzam, Farah F Al-

Mamoori and Ali M Al-Samydai conducted the laboratory work. Talal A Aburjai designed the study, helped in analyzing the results and revised the manuscript. All authors read and approved the final manuscript.

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