

Abstract

The US Food and Drug Administration has recently warned some major retailers to stop selling dietary supplements due to suspected quality issues. There is concern among medical professionals that many supplements may lack purity and/or potency because they are not subject to the same regulations and quality standards as pharmaceuticals. Traditional methods used to determine the content of Dehydroepiandrosterone (DHEA) supplements are HPLC analysis and liquid chromatography–tandem mass spectrometry. An enzyme-linked immunosorbent assay (ELISA) for the detection of DHEA was developed using polyclonal antibodies. The total assay time was less than 70 minutes with a detection limit of 5 ng/ml (ppb). Twenty four commercial DHEA supplements obtained from health food stores and pharmacies were analyzed. These supplements were supplied in tablet or capsule forms with labeled concentrations varying from 10 mg to 300 mg. The ELISA test results indicated that the samples contained about 56% to 138% of their labeled concentrations. The test was evaluated to determine the range of spike and recovery for the method (85-100%). Cross reactivity with other hormones was tested. The results indicate that the ELISA kit developed in this study can be used as a rapid and accurate tool for quantification of DHEA content in supplements.

Introduction

Dehydroepiandrosterone is a precursor to estrogen and androgen hormone production in the human body. DHEA is a steroid hormone produced by the adrenal glands, brain and gonads. The circulating levels begin to decline after age 30. DHEA levels in the body have been studied for their involvement in many different diseases. Low levels of the hormone are found in individuals with hormone and immune disorders, Alzheimer's disease, depression, diabetes, osteoporosis and heart disease to name a few.

The use of this steroid in athletic competition is banned by the World Anti-Doping Code and the NCAA, however many athletes have tested positive for the compound. DHEA increases muscle growth and contributes androgenic effects to those athletes who take it. DHEA is banned from sale without prescription in most countries except in the US where it was exempted from the Anabolic Steroid Control Act of 1990 and 2004.

ELISA is a simple and easy method to quantify levels of small molecules like DHEA, and the method lends itself to use in a manufacturing operation where quality control analysis is needed. In this study a competitive ELISA was developed and evaluated to determine DHEA levels in different supplement formulations. This method was tested on multiple supplements for reactivity and interferences. Cross reactivity to related compounds and the % recovery of DHEA from the method were evaluated.

Table 1. Detecting DHEA in Commercial DHEA Dietary Supplements

DHEA Supplement Samples	Average Concentration DHEA	% Recovery
Neg. Control	NA	---
DHEA 5 ng/ml	4.7 ppb	95
DHEA 30 ng/ml	26.1 ppb	87
DHEA 150 ng/ml	129.8 ppb	87

Methodology

Polyclonal antibody production:

In the development of this assay, DHEA was coupled to a protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. The antiserum was collected monthly.

Sample preparation for ELISA Analysis:

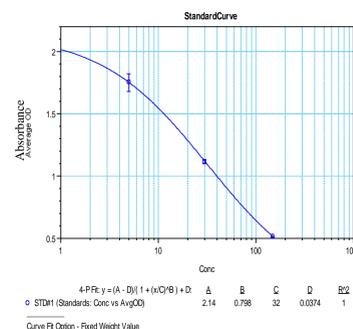
- Dissolve one whole pill in 20 ml of 80% methanol/DI water, soak for at least 30 min at room temperature. Tablets were ground into powder prior to addition of 80% methanol solution.
- Centrifuge at 8,600 x g for 5 minutes.
- Dilute 0.5 ml supernatant with 0.5 ml 80% methanol and mix well using a vortex mixer (10-15 seconds).
- Centrifuge at 8,600 x g for 5 minutes.
- Dilute sample with 10% methanol/DI water.

Assay protocol:

- Add 50 µl of HRP conjugate to each well.
- Add 50 µl of calibrator or sample extract.
- Add 50 µl of antibody solution to the wells and mix gently for 30 seconds.
- Incubate the wells for 30 minutes at room temperature.
- Dump the well contents and wash 4 times with tap water.
- Add 100 µl of substrate solution into each well and incubate for 30 minutes at room temperature.
- Add 100 µl of stop solution and measure absorbance at 450 nm - 650 nm.
- DHEA concentration is derived from the calibration curve multiplied by the total dilution factor used in the sample extract preparation.

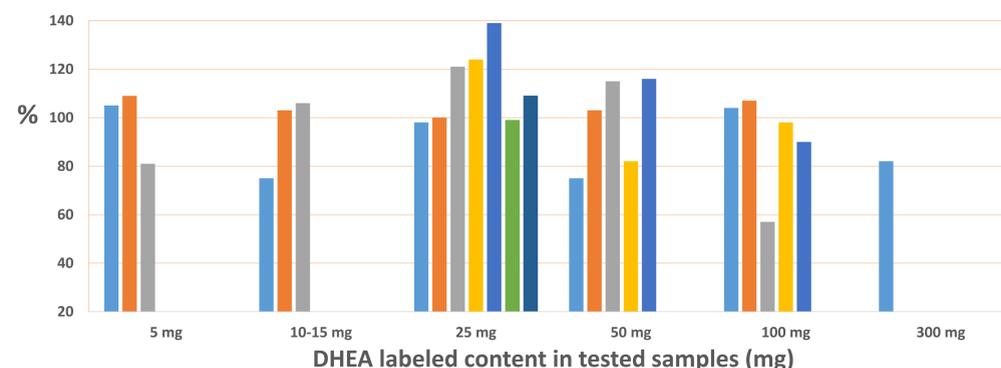
Results

Calibration Curve



Dehydroepiandrosterone (ppb)

Figure 1. Quantitative Analysis Results from 24 Dietary Supplement Samples
DHEA content relative to label concentration (%)



Results

Table 2. Negative Confirmation Test with other Dietary Supplements

Sample	Conc. of spiked DHEA	ELISA
		Recovery DHEA (%)
Blueberry Extract	150 ppb	99.6
Multiple Vitamin	150 ppb	99.1
Vitamin B Complex	150 ppb	104

Table 3. Cross-reactivity with related compounds @ 1c50%

Compound	Cross-reactivity
DHEA	100%
Pregnenolone sulfate	0.01%
17-Hydroxyprogesterone	< 0.01%
17-Hydroxypregnenolone	2.9%

Discussion

A simple and rapid competitive immunoassay was developed for determination of DHEA levels in commercially available dietary supplements. The assay calibration curve covers a range of 5 - 200 ppb, each sample extract was diluted to fall within the calibration range. Results from ELISA analysis of the DHEA compound from the supplements differed slightly from labeled amounts. The DHEA from one manufacturer was at 56% of the labeled concentration and is the lowest of 24 vendors analyzed. The other 23 DHEA supplements from various manufacturers were also tested by ELISA and their results correlated reasonably well with their labeled concentrations (Figure 1). Non-herbal supplements were also analyzed for DHEA concentrations and they were found to be correctly labeled as well. The variety of tablets/capsules included in the testing suggests that the method is a rugged and easy method to monitor the many types of dietary supplements sold by retailers. It can also be an effective tool for supplement manufacturers to incorporate into their quality management programs. These results indicate that the ELISA kit developed in this study (96-well plate format) can be used as a rapid and accurate tool for the determination of the DHEA concentrations in supplements from various manufacturers.

References

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