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Direct Determination of Prednisolone by Derivative UV Spectroscopy

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ABSTRACT

UV Derivative spectra were used for the direct quantitative determination of Prednisolone in absolute ethanol, the quantification was accomplished according to the integrated area under the peaks. The zero-order spectrum of Prednisolone show an absorption band at λ = 242nm, with molar extinction coefficient ε_{max} = 13770 lit. mol^{-1}. cm^{-1}, the determination range was (0.36-50.46) µg/ml with R^2 =0.9998 and relative standard deviation RSD =1.28 %.

The determination ranges were (0.10- 72.09) µg/ml, (0.36-72.09) µg/ml for first and second-order derivatives respectively with R^2 = 0.9999, 0.9998 and RSD = 1.53%, 2.55% respectively.

This indicate a more sensitive and accurate results as compared with the zero-order method. These methods were applied for the direct determination of the PRISOLONE tablets.

التقدير المباشر للبريدنزولون باستخدام طيف المشتقة للاشعة فوق البنفسجية

الملخص

يتضمن البحث طريقة مباشرة لتقدير بريدنزولون في الايثانول المطلق باستخدام طيف المشتقة للأشعة فوق البنفسجية (UV). يعتمد التقدير الكمي المباشر على قياس المساحة تحت الحزمة ضمن مدى معين من الأطوال الموجية. إن الطيف الاعتيادي (zero-order spectra) يظهر حزمة امتصاص عند الطول ألموجي 242 نانوميتر وبمعامل امتصاص مولاري 13770 لتر مول$^{-1}$.سم$^{-1}$. وكان مدى التقدير (0.36-50.4) مايكرو غرام/مللتر وR^2=0.9998 و RSD=1.28%.

أما عند تطبيق التقدير المباشر باستخدام طيف المشتقة الأولى والثانية فقد أظهرت مدى تقدير (0.10-72.09), (0.36-72.09) مايكرو غرام/مللتر على التوالي مع R^2 = 0.9999, 0.9998, RSD = 1.53%, 2.55% على التوالي، مما يدل على زيادة في مدى التقدير وان الطريقة حساسة ودقيقة، وقد استخدمت هذه الطرائق في التقدير المباشر لحبوب البريزولون بنجاح.

INTRODUCTION

Prednisolone is a synthetic steroid that is chemically defined as 11,17,21-trihydroxypregna-1,4-diene-3,20-dione. Its structure is shown in figure 1 (Maffat *et al.*, 2005).

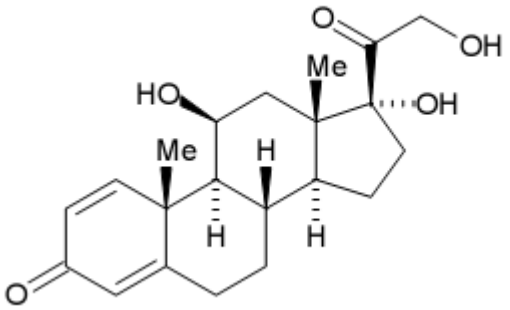

Fig. 1: Structure of Prednisolone, molecular weight =360.4

Prednisolone is a well –known corticosteroid that is used to treat a wide variety of acute and chronic disorders such as arthritis, asthma, allergic diseases (Vogt *et al.*, 2006).

A number of methods were used for the quantitative determination of prednisolone, High-Performance Liquid Chromatography (HPLC) method (Singh and Verma, 2007; Gai *et al.*, 2005; Gorog S., 2004; Ali *et al.*, 2002; Majid *et al.*, 2001; Doppenschmitt *et al.*, 1995; Cheng *et al.*, 1988; Ost *et al.*, 1982), Micellar Electro kinetic Chromatography (MEKC) (Gallego and Arroyo, 2003), Gas Liquid Chromatography (Matin and Amos, 1978), Isotope dilution –Mass Spectrometry (Ost *et al.*, 1982), Chemical – Ionization Mass Spectrometry (Matin and Amos, 1978), FT – Raman Spectroscopy (Mazurek and Szostak, 2005), Photochemical Induced Fluorescence (Coelho and Aucelio, 2006), Polarographic Catalytic Wave (Guo *et al.*, 2002) and Second Derivative Spectrophotometry (Singh and Verma, 2007).

In this work the direct quantification of Prednisolone was accomplished according to the integrated area under the peaks within a range of wavelength, the peak area measurements are often found to be more reliable than peak height measurement (Singh and Verma, 2007).

EXPERIMENTAL

Chemicals and solutions

1. Pure Prednisolone (SDI):

   A stock solution of (10^{-3}) M was prepared by dissolving (0.0036) gm of pure Prednisolone in 10 ml absolute ethanol, then by proper dilution, other less concentrated solutions were prepared and their spectra recorded.

2. PRISOLONE Tablets (SDI, Iraq, Prednisolone 5 mg):

   From 10 grinded tablets a weight equivalent to (0.0036) gm of pure Prednisolone was dissolved in 10 ml absolute ethanol to prepare (10^{-3}) M of Prednisolone drug solution other concentrations were prepared from this solution by proper dilution with absolute ethanol and their spectra were recorded.

3. Absolute ethanol (GCC, Analyt, UK).

INSTRUMENTATION

Shimadzu UV-Visible spectrophotometer model UV-1650 PC, connected to a computer with pentium 4 processor, The optimized conditions for spectrophotometic measurements were derivative modes ^{1}Dr ($d^1A/d\lambda^1$), ^{2}Dr($d^2A/d\lambda^2$), scan speed fast, slit width 2nm, derivative UV spectra were recorded over wavelength range of (200-400) nm, using (1×1×3) cm matched quartz cells.

RESULT AND DISCUSSIN

The zero, first and second order derivative spectra of pure Prednisolone in absolute ethanol for a series of different concentrations (0.3×10^{-6}-200×10^{-6}) M, were recorded (figure 2). The zero-order spectrum shows an absorption at λ = 242 nm with a molar extinction coefficient of about 13770 lit. mol^{-1}. cm^{-1}. The plot of the recorded absorbance against the molar concentration of pure Prednisolone result in a straight-line obeying the Beer's-Lambert law within a concentration range of (1×10^{-6}-140×10^{-6}) M, and a determination range of (0.36-50.46) μg\ml, with R^2 = 0.9998, and RSD = 1.28 % (Table 1, figure 3).

Abs

Wavelength/nm

Fig. 2: The UV absorption (zero (…..), first (—), second (—)) order derivative spectra of (140×10^{-6}) M of pure Prednisolone solution

Table 1:The absorbance of the zero –order spectra at λ =242 nm for different concentration of pure Prednisolone in absolute ethanol.

Molar concentration $\times 10^{-6}$	Absorbance
1	0.02
2	0.034
3	0.047
5	0.075
7	0.103
10	0.145
30	0.430
50	0.693
70	0.961
90	1.252
100	1.398
110	1.538
120	1.669
130	1.779
140	1.916

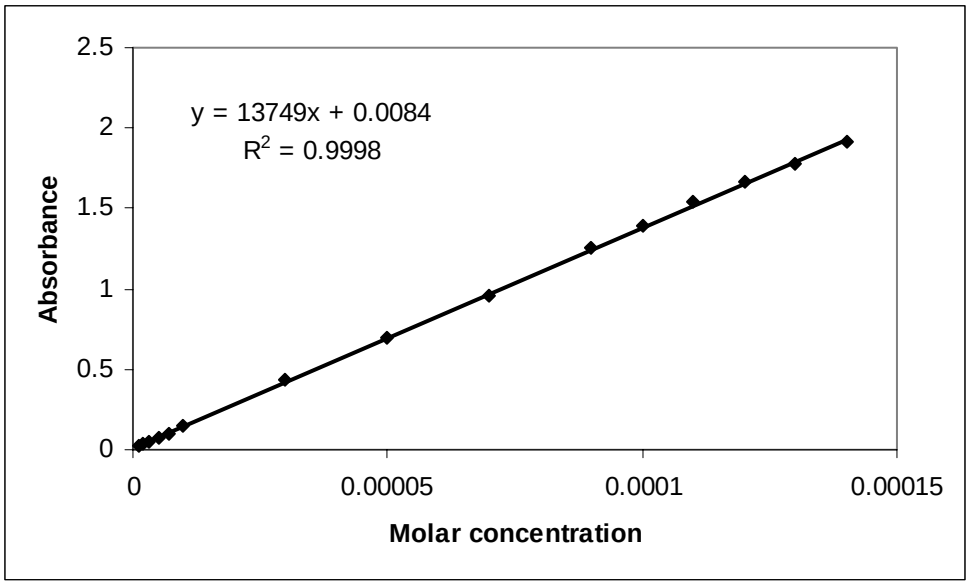

Fig. 3: The calibration curve of the zero –order spectra of pure Prednisolone solutions

The applications were accomplished by recording the zero-order spectra of PRISOLONE Tablets (SDI, Iraq, Prednisolone 5 mg) which resemble the spectra of pure Prednisolone, at λ = 242 nm at different concentrations of Prednisolone in PRISOLONE Tablets and the absorbance were plotted against the molar concentration. The result was a

straight-line between the concentration ranges (1×10^{-6}-130×10^{-6}) M with $R^2 = 0.9999$, and the recovery percent were estimated (Table 2).

Table 2: The absorbance of the zero-order spectra at λ = 242 nm for different concentration of Prednisolone in PRISOLONE Tablets solutions.

Molar Concentration Taken $\times10^{-6}$	**Absorbance***	**Molar Concentration Found $\times10^{-6}$**	**Recovery%**	**Relative Error%**
10	0.136	9.2	92.806	-7.193
50	0.677	48.6	97.258	-2.742
100	1.380	99.7	99.759	-0.240

**Mean of five readings*

The first-order derivative spectrum of pure Prednisolone shows a positive peak at λ= (224-242) nm, crossing the zero-axis at λ = 242 nm and a negative peak at λ = (242-340)nm (Fig. 2). The quantitative determination of pure Prednisolone was accomplished through plotting of a calibration curve between the integrated area under the negative peak against the molar concentration of pure Prednisolone solutions. The result was a straight- line obeying the Beer's-Lambert law with a determination limit of (0.10-72.09)µg\ml, $R^2 = 0.9999$ and RSD = 1.53 % (Table 3, fig. 4).

Table 3: The integrated areas under the negative peak λ =(226-268) nm of the first –order derivative spectra of pure Prednisolone solutions at different concentrations.

Molar Concentration $\times10^{-6}$	Integrated Area
0.3	0.006
0.7	0.011
1	0.016
2	0.032
3	0.045
5	0.071
7	0.096
10	0.135
30	0.398
50	0.64
70	0.888
90	1.157
100	1.288
110	1.417
120	1.537
130	1.641
140	1.767
150	1.892
160	2.019
170	2.146
180	2.271
190	2.397

200	2.524

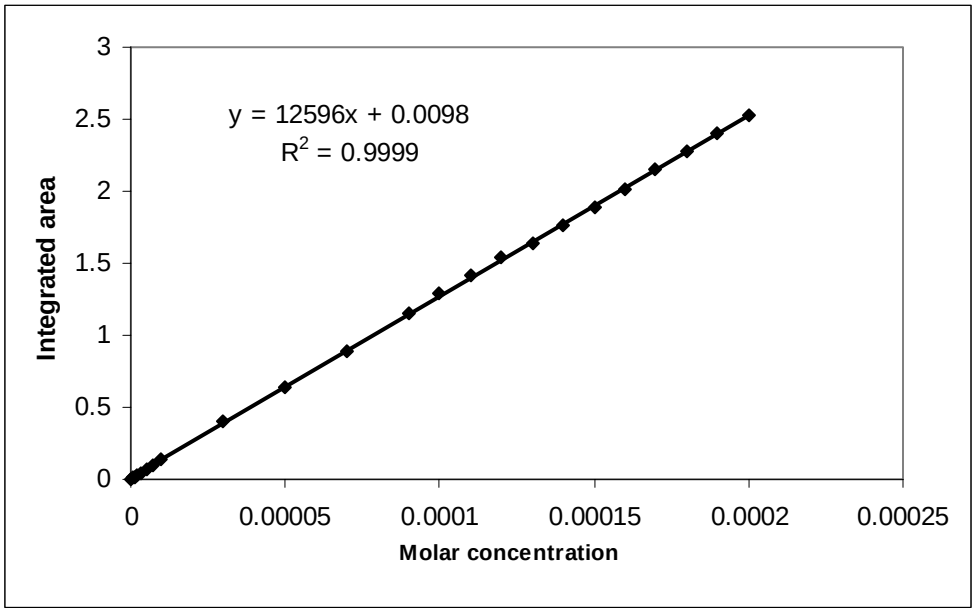

Fig. 4: The calibration curve of the first –order derivative spectra of pure Prednisolone solutions

The first-order derivative spectrum for PRISOLONE drug solutions recorded at different concentrations and the area under the negative peak at λ = (242-340)nm was integrated and the recoveries were estimated (Table 4).

Table 4: The integrated area under the negative peak of the first-order derivative spectra at λ = (242-340) nm for different molar concentration of PRISOLONE Tablets solutions.

Molar Concentration Taken $\times 10^{-6}$	**Integrated Area***	**Molar Concentration Found $\times 10^{-6}$**	**Recovery%**	**Relative Error%**
10	0.136	10.0	100.190	+0.190
50	0.634	49.5	99.110	-0.889
100	1.278	100.6	100.682	+0.682

⋆*Mean of five readings*

The spectra of the second-order derivative for different concentrations of pure Prednisolone shows a negative peak at λ = (226-268) nm (Fig. 2), the integrated area under this peak were plotted against the molar concentrations the result is a straight-line relationship obeying the Beer's-Lambert law with a determination range of (0.36-72.09) μg\ml, R^2 = 0.9998, and RSD = 2.55%. (Fig. 5)

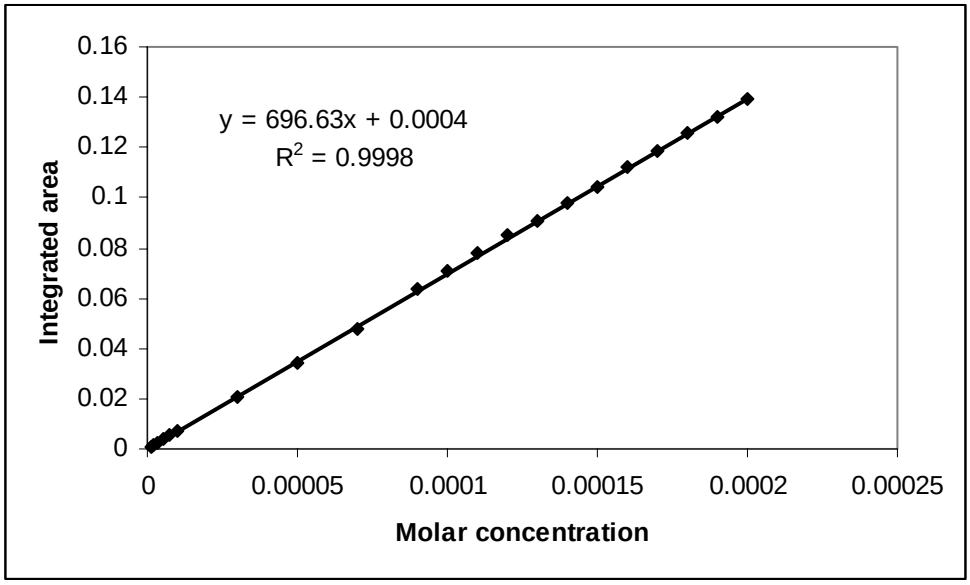

Fig. 5: The calibration curve of the second-order derivative spectra of pure Prednisolone solution

The second-order derivative spectra of a series for PRISOLONE drug solution show a negative peak at λ = (226-268) nm. The areas are integrated and the recoveries were estimated (Table 5)

Table 5: The integrated area under the negative peak of the second-order derivative spectra at λ = (226-268) nm for different concentration of PRISOLONE drug solutions.

Molar Concentration Taken $\times 10^{-6}$	**Integrated Area***	**Molar Concentration Found $\times 10^{-6}$**	**Recovery%**	**Relative Error%**
10	0.007	9.4	94.742	-5.258
50	0.034	48.2	96.464	-3.536
100	0.064	91.2	91.296	-8.703

⋆Mean of five readings

CONCLUSIONS

The first-order derivative method was the best method for the quantitative determination of pure Prednisolone as compared with the zero and second-order methods. The same results proved fruitful for the determination of Prednisolone in the PRISOLONE Tablets (the best recovery percent and the minimum percent of error). The determination range of Prednisolone was improved by using derivative spectrophotometry which compared than the normal spectra.

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