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Research Article

A Comparative Study of UV-Spectrophotometry and First-Order Derivative UV-Spectrophotometry Methods for the Estimation of Diazepam in Presence of Tween-20 and Propylene Glycol

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Abstract. Nonionic surfactants like polysorbates (Tweens) and co-surfactant like propylene glycol are used in pharmaceutical dosage forms, like microemulsion of diazepam. These additives interfere significantly with the estimation of diazepam by UV spectrophotomery method. The aim of this work was to develop a first-order derivative UV-spectrophotometry method that can estimate diazepam in presence of Tween-20 and propylene glycol. The experimental results clearly suggested that, in comparison with the UV-spectrophotometry method, the first-order derivative UV-spectrophotometry is a simple method to estimate diazepam with sufficient accuracy, specificity, and precision even in the presence of 282-times Tween-20 and 2,072-times propylene glycol.

KEY WORDS: derivative spectrophotometry; diazepam; first-order; propylene glycol; Tween-20.

INTRODUCTION

Diazepam, a benzodiazepine derivative (7-chloro-1,3-dihydro-1-methyl-5-phenyl 2H-1,4-benzodiazpein) [CAS: (439-14-5)], is used for managing convulsion/epilepsy, pre-operative medication, and sedation of short time duration and to relieve generalized anxiety and sleep disorder (1). Diazepam is generally available as conventional tablets and intravenous injection. Official compendia suggest liquid chromatographic method for estimation of diazepam in tablets and injectables. Besides the official methods, numerous analytical techniques like gas chromatography (2), HPLC (3,4), capillary electrophoresis (5), TLC–densitometry (6), electrochemical method (7), and flow injection fluorometry (8) have been reported for analysis of the drug. Spectrophotometry methods (9) are also available for assay of diazepam in conventional pharmaceutical dosage forms.

During the last few decades, a great interest has been seen in the development of various novel drug delivery systems. In many of these formulations, surfactants like polysorbates (Tweens) and co-surfactants like propylene glycol and polyethylene glycol (PEG) are used. For instance, a patented method disclosed the use of Tweens, propylene glycol, and PEG in intranasal microemulsion dosage form of diazepam (10). However, as those additives exhibit considerable absorbance at the wavelength of maximum absorbance (λ_{max}) of diazepam UV-spectrometry method cannot be used to estimate diazepam accurately in their presence.

This paper presents first-order derivative UV-spectrophotometry as an accurate, precise, and simple method in comparison to UV-spectrophotometry method for the estimation of diazepam in presence of Tween-20 and propylene glycol.

MATERIALS AND METHODS

Materials

Diazepam (IP 2007) was obtained as gift sample from Torrent Pharmaceutical Ltd., India. It contains not less than 98.5% and not more than 101.0% diazepam, calculated with reference to the dried substance. Tween-20, propylene glycol (SD FINE-CHEM Limited, Mumbai, India), methanol AR, potassium–dihydrogen–phosphate GR (Merck Ltd., Mumbai, India), and all other reagents were obtained commercially and used as received. Bi-distilled water was used to prepare phosphate buffer solution (pH 7.4; 11), standard solution of drug, and other reagents.

Apparatus

Spectrophotometric measurements were performed on a Varian Cary-50, Bio Spectrophotometer (Varian, Inc.) under the following optimized working settings—scan range, 200 to 400 nm; slit width, 1.5 nm; average time interval, 0.5 s; data interval, 2 nm; scan rate, 240 nm/min.

Derivative spectra were obtained mathematically from the absorbance spectrum with FindGraph, Version 1.924.

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Methods

Construction of Absorbance Spectrum of Diazepam in Phosphate Buffer Solution (pH 7.4)

Accurately weighed amount of diazepam was dissolved in 5 ml methanol and diluted to 100 ml with phosphate buffer solution. This drug solution was diluted five times to get a stock solution having concentration of 25 μg/ml. Aliquots from this stock solution were further diluted to 25 ml with buffer solution to get standard solutions having concentrations from 1 to 9 μg/ml. Each of these solutions was scanned from 200 to 400 nm against phosphate buffer as blank. Standard solutions were prepared three times a day from the same stock solution (25 μg/ml) and subsequently scanned to get the absorbance value (A) at 2-nm interval. The entire experiment was replicated for three consecutive days.

Construction of First-Order Derivative Spectrum of Diazepam in Phosphate Buffer Solution (pH 7.4)

The absorbance values (A) at various wavelengths (λ; ranging from 200 to 400 nm) of each standard solution was used to calculate the slope of the tangent $\frac{(dA)}{d\lambda}$ at each data point and plot of $\frac{dA}{d\lambda}$ against λ generated the corresponding first-order derivative spectra.

Construction of Calibration Curves

Calibration curves were constructed by linear regression analysis, using the method of least square. The absorbance values (A) at 230 nm and $\frac{dA}{d\lambda}$ values at 260 nm of five standard solutions were used to construct the calibration curves for UV-spectrophotometry and first-order derivative UV-spectrophotometry methods, respectively.

Stability of Diazepam in Phosphate Buffer Solution (pH 7.4)

The standard solutions of diazepam in phosphate buffer prepared for the calibration curves were analyzed by both methods at 0, 2, 4, 6, 24, 48, and 72 h after preparation. The behavior of diazepam remained unchanged during the whole period of study.

Recovery Analysis

Graded volumes (1.5 to 4 ml) of a 20-μg/ml stock solution of diazepam in phosphate buffer, 0.1 ml 100 mM Tween-20 solution, and 0.08ml propylene glycol were taken in 10-ml volumetric flasks, and the volumes were made up to the mark with buffer solution. Each solution was scanned in the range of 200 to 400 nm. The absorbance values (A) at 230 nm and $\frac{dA}{d\lambda}$ values at 260 nm were used to calculate the concentration of diazepam in presence of 1 mM Tween-20 and 0.8% (v/v) propylene glycol from the respective calibration curves. The recovery analysis was triplicated each day for three consecutive days.

Determination of Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) of diazepam were determined in presence of 1 mM Tween-20 and 0.8% (v/v) propylene glycol. 0.1 ml Tween-20 and 0.08 ml propylene glycol were diluted to 10 ml with phosphate buffer. The absorbance (A) and $\frac{dA}{d\lambda}$ value of this solution at 230 nm and 260 nm, respectively, were determined against phosphate buffer as blank. These measurements were replicated for 12 times with freshly prepared sample, and the standard deviation (SD) of the determined parameters (A and $\frac{dA}{d\lambda}$) were calculated. Then, the respective calibration curves were used to estimate the LOD and LOQ from the following equations.

$$LOD = \frac{3 \times SD}{slope\ of\ calibration\ curve},$$

$$LOQ = \frac{10 \times SD}{slope\ of\ calibration\ curve}$$

RESULTS AND DISCUSSION

The wavelength of maximum absorbance (λ_{max}) of diazepam in phosphate buffer solution (pH 7.4) was found to be 230 nm (Fig. 1). Presence of 1 mM Tween-20 and 0.8% (v/v) propylene glycol in standard solution of drug having 3 μg/ml concentration caused λ_{max} to shift from 230 to 228 nm and increased the absorbance value by 38.40%. Such interference was observed throughout the total range of drug concentration studied, although the level of interference decreased with increase in drug concentration (Fig. 2).

The first-order derivative spectra of diazepam consisted of one positive peak at 224.1 nm and two major negative peaks at 238 and 260 nm (Fig. 3). Though the first-order derivative spectrum of diazepam in the presence and absence of 1 mM Tween-20 and 0.8% (v/v) propylene glycol were not superimposed; the change in $\frac{dA}{d\lambda}$ value at 260 nm was much smaller than the corresponding change in A value at 230 nm. For example, the $\frac{dA}{d\lambda}$ value at 260 nm of 3 μg/ml diazepam solution was increased by about 10% due to the presence of 1 mM Tween-20 and 0.8% (v/v) propylene glycol (Fig. 4). This deviation (10%) was much less than that of absorbance value at 230 nm (38.4%). Moreover, as the concentration of diazepam was increased, the effect of Tween-20 and propylene glycol on $\frac{dA}{d\lambda}$ value at 260 nm reduced more sharply than absorbance (A) value at 230 nm.

According to ICH guidelines, the essential estimates to establish linearity are (1) correlation coefficient, (2) Y-intercept, (3) slope of regression line, and (4) residual sum of squares. The statistical parameters for the calibration curves obtained by the two methods have been shown in Table I. From the comparative data, it was obvious that the first-order derivative UV-spectrophotometry method provided a calibration curve with r^2 value closer to unity, Y-intercept value closer to 0, and residual sum of square about 30,000 times smaller than that obtained from UV-

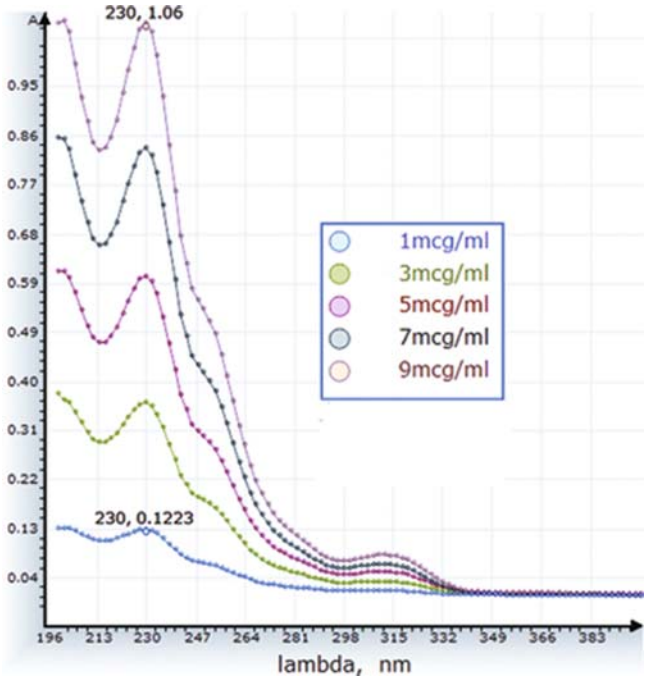

Fig. 1. Absorbance spectra of five standard solutions of diazepam in phosphate buffer (pH 7.4). The legends for different concentrations are shown as *inset* in the figure

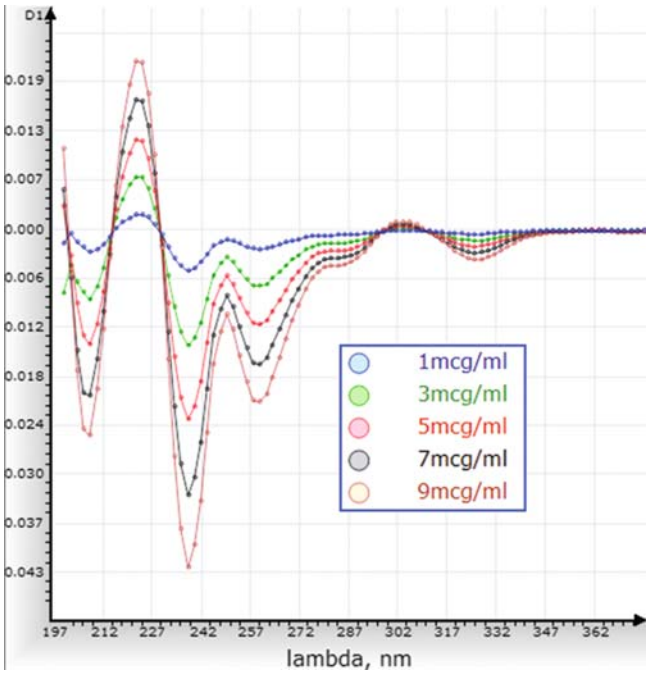

Fig. 3. First-order derivative spectra of different standard solutions of diazepam in phosphate buffer (pH 7.4). The legends for different concentrations are shown as inset in the figure

spectrophotometry method. Thus, the concentration of diazepam in phosphate buffer solution was much better correlated linearly with $\frac{dA}{d\lambda}$ value at 260 nm than the absorbance value at 230 nm.

Since the 95% confidence interval of intercept (Table I) did not include the null point, the calibration curve for first-order derivative spectroscopy method did not pass through the origin. This may be due to "systemic error".

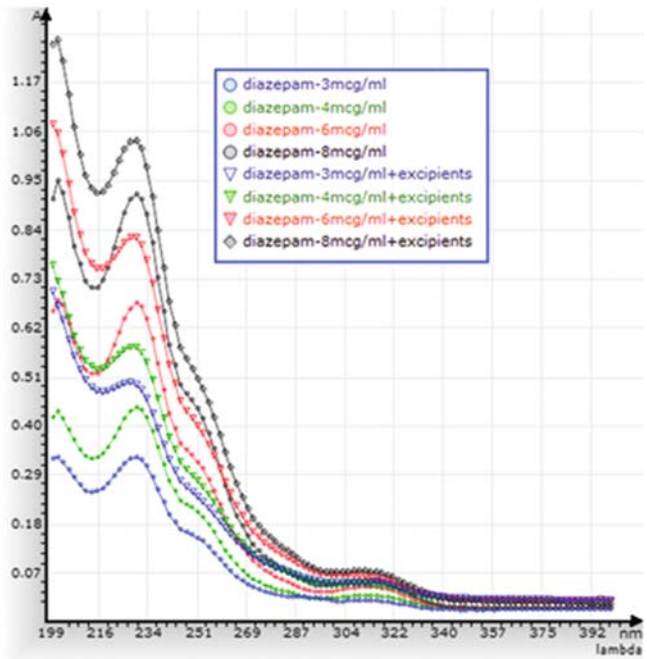

Fig. 2. The effect of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol on the absorbance spectra of standard solutions of diazepam in phosphate buffer (pH 7.4). The legends for absorbance spectrum of different standard solutions of diazepam in the absence and presence of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol are shown as *inset* in the figure

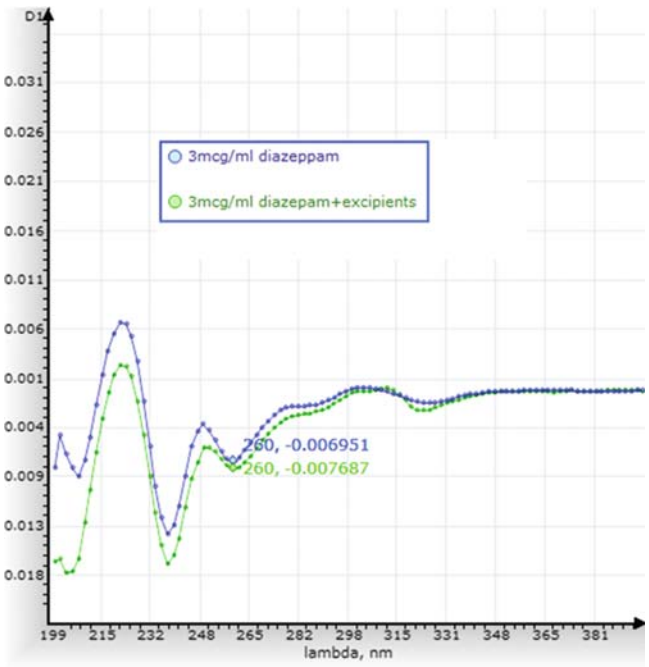

Fig. 4. The effect of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol on the first-order derivative spectrum of 3 μg/ml solution of diazepam in phosphate buffer (pH 7.4). The legends for absorbance spectrum of 3 μg/ml solution of diazepam in the absence and presence of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol are shown as *inset* in the figure

Table I. Spectral Data of Diazepam in Phosphate Buffer (pH 7.4)

Parameters	Values	
	UV spectrophotometric method	First-order derivative UV spectrophotometric method
λ (nm) at which standard curve was constructed	230	260
Regression equation	Absorbance (A)=0.1174×concentration (μg/ml)−0.006038	D_1=−0.00238×concentration (μg/ml)−1.15×10^{-4}
Intercept(a)	0.006038	-1.15×10^{-4}
Slope(b)	0.1174	−0.00238
S_a[a]	0.003810	2.45×10^{-5}
S_b[b]	0.0006633	4.26×10^{-6}
$\pm tS_a$[c]	−0.01373 to 0.001651	0.00006610 to 0.0001649
$\pm tS_b$[d]	0.1161 to 0.1187	−0.002388 to −0.002371
Correlation coefficient (r^2)	0.9986	0.9999
$S_{y.x}$[e]	0.01259	0.00008087
Residual sum of squares	1.105×10^{-5}	3.566×10^{-9}

[a] Standard deviation of intercept
[b] Standard deviation of slope
[c] 95% Confidence interval of intercept
[d] 95% Confidence interval of slope
[e] Standard error of correlation coefficient

Table II. Statistical Result of Recovery Analysis

Analytical methods	Diazepam concentration (μg/ml)	Average predicted concentration (μg/ml)	Percent recovery	Percent bias	RSD[a] (%) of inter-day variation
UV-spectrophotometry	3	4.122489	137.416300	37.416300	3.005633
	4	4.969810	124.245300	24.245250	0.1395995
	6	6.941591	115.693200	15.693180	1.581322
	8	8.800074	110.000900	10.000930	1.67945
First-order derivative	3	3.254128	108.470900	8.470933	0.5707101
UV-spectrophotometry	4	4.151340	103.783500	3.783500	0.450889
	6	6.004737	100.078900	0.078950	1.005277
	8	7.860795	98.259950	−1.740062	0.3703839

[a] Relative standard deviation, represent intermediate precision

Table III. Repeatability of the Analytical Methods

Analytical methods	Diazepam concentration (μg/ml)	Percent RSD		
		Day 1	Day 2	Day 3
UV-spectroscopy	3	0.16876	0.15979	0.16767
	4	0.10295	0.10268	0.10291
	6	0.04977	0.04825	0.04926
	8	0.01973	0.01908	0.01929
First-order derivative UV-spectroscopy	3	0.064356	0.058048	0.06499
	4	0.040654	0.040528	0.040311
	6	0.020909	0.021243	0.020837
	8	0.005362	0.005362	0.005321

The recovery analysis of diazepam at four concentration levels in presence of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol have been shown in Table II. It was evident that, while the range of recovery varied from 98% to 108.5% with a bias of −2% to 8.5% by the first-order derivative UV-spectrophotometry method, the same varied from 110% to 137% with a bias of 10% to 37% by the UV-spectrophotometry method. Thus, the first-order derivative UV-spectrophotometry method was more accurate than the UV-spectrophotometry method.

The first-order derivative UV-spectrophotometry method was found to quantify diazepam at 4 μg/ml concentration in the presence of as much as 282 times Tween-20 and 2,072 times propylene glycol with approximately 104% recovery (*i.e.*, 4% bias) and 0.45% relative standard deviation (RSD). On the other hand, although percent RSD was 0.1359, the bias was approximately 24.25% when quantification was done with UV-spectrophotometry method. High percentage of bias suggests that, in comparison to UV-spectrophotometry method, first-order derivative UV-spectrophotometry method is specific enough to quantify diazepam with acceptable limit of accuracy.

Furthermore, the intermediate precision (ruggedness) of first-order derivative UV-spectrophotometry method was higher as the inter-day percent RSD values (≤ 1) were less than that of UV-spectrophotometry method (percent RSD ≤ 3).

The intra-day relative standard deviation (percent RSD), representing the repeatability of the analytical methods, are shown in Table III. A small value represents better repeatability. Hence, in comparison to UV-spectrophotometry method, the first-order derivative UV-spectrophotometry method had better repeatability.

The LOD and LOQ for the UV-spectrophotometry method were found to be 0.153 μg/ml and 0.389 μg/ml, respectively. The values of the same parameters for the first-order derivative UV-spectrophotometry method were 0.084 μg/ml and 0.168 μg/ml. Thus, in presence of 1 mM Tween-20 and 0.8% (*v*/*v*) propylene glycol, the first-order derivative UV-spectrophotometry method was found to detect and quantify diazepam at much lower concentration than the UV-spectrophotometry method.

CONCLUSION

UV-spectrophotometry method appeared to be unsuitable for the estimation of diazepam in presence of Tween-20 and propylene glycol used as additives in pharmaceutical formulations. However, the first-order derivative UV-spectrophotometry method can be used to measure diazepam in the presence of those additives with sufficient accuracy and precision. The proposed method is simple, rapid, and economical for routine analysis of diazepam in pharmaceutical laboratories.

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