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# Determination of Specific Absorbance ( $A_1^1$ ) for Zaleplon (Sonata<sup>®</sup>) by Spectrophotometry

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## Abstract

Specific absorbance ( $A_1^1$ ) is the maximum absorbance of a 1% solution over a 1-cm path length as measured by a spectrophotometer. If a reliable  $A_1^1$  determination is available for a drug, it provides an extremely useful tool for the quantitative verification of a stock drug solution. Zaleplon was introduced into the market in 1999, and although the drug has been available for 10 years,  $A_1^1$  has not been published in the literature. Zaleplon's  $A_1^1$  was experimentally determined by a spectrophotometer at 229 nm in aqueous acid and verified with three independent external sources to be 1186 (1042–1262;  $n = 18$ ). The experimentally determined  $A_1^1$  of zaleplon is beneficial to a toxicology laboratory to verify the quantitative accuracy of a drug solution prior to its use in casework.

## Introduction

The identification and quantitation of any drug within a toxicology laboratory requires that the analyst obtain a known analytical standard from a reputable source, verify the drug and/or concentration, and compare the verified standard to the unknown before reporting such in casework. There are several options for obtaining an analytical standard, one of which is requesting the reference powder directly from the pharmaceutical manufacturer with a known certificate of authenticity. Other possibilities include purchasing the purified powder or drug in solution at a known concentration from a commercial source, purifying the drug from a pill, or obtaining the drug powder/solution from another toxicology laboratory. Unless the analytical solution is accompanied by a certificate of authenticity, the drug or drug solution will need to be quantitatively verified by the laboratory before being put into production. Although there are several ways to accomplish verification of concentration, comparison of the specific absorbance of a drug that is determined by spectrophotometry to an established literary value is a quick and easy method.

The specific absorbance, also known as a molar absorptivity

coefficient or extinction coefficient, symbolized as  $A_1^1$ , is described by *Clarke's Analysis of Drugs and Poisons* as the absorbance of a 1% solution (w/v) in a cell of 1-cm path length (1).  $A_1^1$  is widely used in analytical chemistry, and the practical usefulness of this value depends on a number of factors. These include the state of purity of the substance, the solvent conditions originally used to establish the reference data, and the precise conditions employed in the reference instrument (1).  $A_1^1$  of any drug can be experimentally determined by a spectrophotometer and expressed as a concentration of g/100 mL or 10 mg/mL (1). Hence, a drug solution can be quantitatively determined by spectrophotometry as long as  $A_1^1$  is well-established and can be relied upon.

Many new drugs have emerged within the last 10 years where an  $A_1^1$  literary value has not been determined or the value simply has not been published. Zaleplon (Figure 1) is a relatively new drug that emerged on the market in 1999 as a sedative hypnotic for short-term insomnia (2). Although the drug has been in existence for many years,  $A_1^1$  has not been established in the literature, and the authors felt the determination of this value was very important for the forensic toxicology community. Therefore, the objective of this paper is to describe the process of determining  $A_1^1$  for zaleplon.

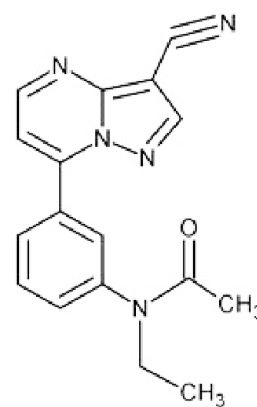


Figure 1. Chemical structure of zaleplon.

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## Experimental

### Materials

The analytical reference material of zaleplon (Lot # CL 284846) was provided by Wyeth-Ayerst Laboratories (Philadelphia, PA) and prepared as a 1.0 mg/mL solution in methanol (MEOH). Additional solutions of zaleplon (1.0 mg/mL MEOH) were purchased from Cerilliant (Round Rock, TX) and graciously provided by two other laboratories. The San Francisco Office of the Chief Medical Examiner (SFOCME, San Francisco, CA) provided a 1.0 mg/mL zaleplon MEOH solution prepared from an analytical reference material purchased from Sigma Aldrich (St. Louis, MO), and the Orange County Sheriff's Department (OCSD, Santa Ana, CA) prepared a 1.0 mg/mL zaleplon MEOH solution from reference material provided by Wyeth-Ayerst Laboratories (Lot # A97D022). All stock solutions of zaleplon were stored in the freezer, and the reagents were analytical-grade and purchased from various vendors.

### Methodology

The experimental design consisted of determining the wavelength for maximum absorbance of zaleplon in MEOH and in 0.1 N hydrochloric acid (HCl) using a Beckman DU 520 Spectrophotometer (Fullerton, CA). In order to determine  $A_i$ , the 1.0 mg/mL zaleplon solutions were adjusted to 0.005, 0.010, and 0.020 mg/mL to be within a measurable absorbance range (less than 3.0) for the spectrophotometer. The  $A_i$  was calculated by measuring the maximum absorbance for zaleplon at three concentrations in 0.1 N HCl (prepared daily) in duplicate analyses over a three-day period ( $n = 18$ ).

## Results

The maximum wavelength of zaleplon was determined to be 231 nm in MEOH and 229 nm in aqueous acid (Figure 2). Absorbance measurements (229 nm, aqueous acid) for 0.005, 0.010, and 0.020 mg/mL, diluted from a 1.0 mg/mL solution of zaleplon prepared in 2009, were performed in duplicate over three days, and the corresponding calculated  $A_i$  values are indicated in Table I. In addition, a 1.0 mg/mL zaleplon solution originally prepared in 2001 was subjected to the same protocol and reflected in Table II. Verification was accomplished with absorbance measurements experimentally determined in diluted concentrations of a 1.0 mg/mL zaleplon solution purchased from Cerilliant as well as solutions ob-

tained from SFOCME and OCSD. The absorbance was measured at the same concentrations in duplicate for a single day. The results are listed in Table III.

## Discussion

Zaleplon concentrations of 0.005, 0.010, and 0.020 mg/mL were analyzed in duplicate over three days ( $n = 18$ ) in order to determine inter- and intrarun precision for a 1.0 mg/mL zaleplon solution prepared in 2009. The coefficient of variation (CV) for inter- and intrarun precision was less than 3% and 5%, respectively. The specific absorbance of the 1.0 mg/mL zaleplon solution prepared in 2009 ranged from 1042 to 1262 ( $n = 18$ ) and averaged 1186 with a median of 1196.

The zaleplon solution prepared in 2001 had an  $A_i$  that ranged from 1178 to 1381 ( $n = 18$ ) and averaged 1268 with a median of 1260. The 2001 drug solution had a slightly higher  $A_i$ , which was probably due to the evaporation of the MEOH over time,

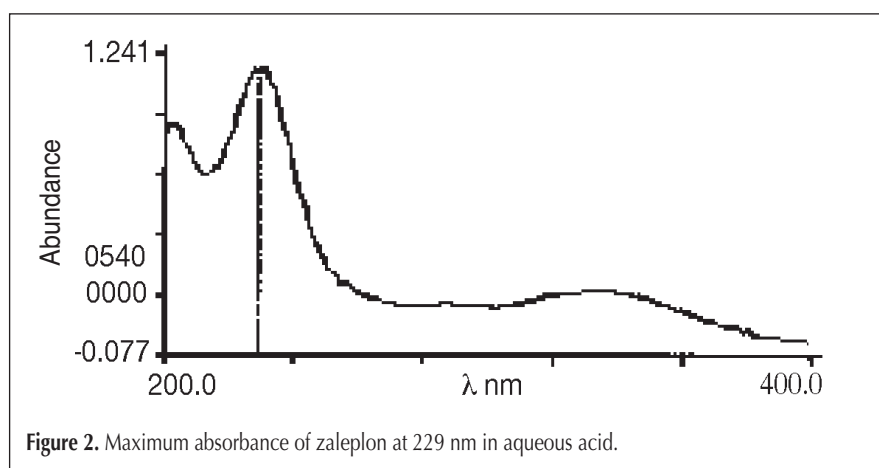


Figure 2. Maximum absorbance of zaleplon at 229 nm in aqueous acid.

Table I. Absorbance Measurements and Calculated  $A_i$  Values for 0.005, 0.010, and 0.020 mg/mL\*

Day	0.005 mg/mL		0.010 mg/mL		0.020 mg/mL	
	Absorbance	$A_i$	Absorbance	$A_i$	Absorbance	$A_i$
1	0.569	1138	1.181	1181	2.359	1180
	0.572	1144	1.217	1217	2.356	1178
2	0.521	1042	1.232	1232	2.451	1226
	0.558	1116	1.225	1225	2.447	1224
3	0.568	1136	1.262	1262	2.369	1185
	0.604	1208	1.229	1229	2.447	1224
Average	0.565	1131	1.224	1224	2.405	1202
Median	0.569	1137	1.227	1227	2.408	1204
<b>Grand average <math>A_i</math></b>		<b>1186</b>				

\* Measured at 229 nm in aqueous acid for the zaleplon solution prepared in 2009 at the Los Angeles County Department of Coroner (1.0 mg/mL).

**Table II. Absorbance Measurements and Calculated  $A_1$  Values for 0.005, 0.010, and 0.020 mg/mL\***

Day	0.005 mg/mL		0.010 mg/mL		0.020 mg/mL	
	Absorbance	$A_1$	Absorbance	$A_1$	Absorbance	$A_1$
1	0.627	1254	1.263	1263	2.430	1215
	0.666	1332	1.381	1381	2.629	1315
2	0.611	1222	1.261	1261	2.501	1251
	0.603	1206	1.244	1244	2.468	1234
3	0.589	1178	1.317	1317	2.572	1286
	0.642	1284	1.320	1320	2.571	1259
Average	0.623	1246	1.298	1298	2.520	1260
Median	0.619	1238	1.290	1290	2.509	1255
<b>Grand average <math>A_1</math></b>		<b>1268</b>				

\* Measured at 229 nm in aqueous acid for the zaleplon solution prepared in 2001 at the Los Angeles County Department of Coroner (1.0 mg/mL).

**Table III. Absorbance Measurements and Calculated  $A_1$  Values for 0.005, 0.010, and 0.020 mg/mL\***

Source	0.005 mg/mL		0.010 mg/mL		0.020 mg/mL		Average $A_1$
	Absorbance	$A_1$	Absorbance	$A_1$	Absorbance	$A_1$	
Cerilliant	0.497	994	1.223	1223	2.474	1237	1148
	0.503	1006	1.195	1195	2.471	1236	
SFOCME	0.499	998	1.125	1125	2.425	1213	1114
	0.506	1012	1.155	1155	2.360	1180	
OCSD	0.550	1100	1.189	1189	2.318	1159	1162
	0.551	1102	1.214	1214	2.419	1210	
<b>Grand average <math>A_1</math></b>		<b>1141</b>					

\* Measured at 229 nm in aqueous acid for the zaleplon solution prepared by external sources (1.0 mg/mL).

thus concentrating the drug in solution. However, the difference in the  $A_1$  values of zaleplon solutions prepared in 2009 and 2001 was only 6.9%, demonstrating that zaleplon in MEOH kept in a freezer over a time period of eight years had excellent stability.

Verification of zaleplon's  $A_1$  1186 was accomplished with three separate external sources of 1.0 mg/mL solutions. The experimentally determined  $A_1$  for Cerilliant, SFOCME, and OCSD solutions averaged 1148 (994–1237,  $n = 6$ ), 1114 (998–1213,  $n = 6$ ), and 1162 (1100–1214,  $n = 6$ ), respectively. In total, the average  $A_1$  of the three external zaleplon sources was 1141 (994–1237,  $n = 18$ ) and was within 96% of the determined  $A_1$  1186 of zaleplon.

According to Moffate's *Clarke's Isolation and Identification of Drugs*, the letter "a" listed after  $A_1$  is defined as a mean value based on several reported figures, all of which lie within a

range of  $\pm 10\%$  of the mean, and a letter "b" is defined as a single reported value of unknown reliability (1). Given the presented analytical data, the letter "a" should be denoted after  $A_1 = 1186$  of zaleplon.

Zaleplon's  $A_1$  1186 is quite large in comparison to other drugs commonly encountered in a toxicology laboratory. In aqueous acid, methamphetamine at 257 nm has an  $A_1$  of 12a, and methadone at 292 nm has an  $A_1$  of 18a, whereas diazepam at 242 nm has an  $A_1$  of 1020a, and temazepam at 237 nm has an  $A_1 = 980b$  (1). In the authors' experience, the larger the  $A_1$  value, the more reproducible and reliable this value will be when determining it from a commonly made 1.0 mg/mL drug solution.

## Conclusions

The specific absorbance of zaleplon was experimentally determined by spectrophotometry and verified by three external sources. Any future literature can cite zaleplon measured at 229 nm in aqueous acid as  $A_1 = 1186a$ . In comparison of the  $A_1$  values of 2001 and 2009 drug solutions, there was minimal difference, thus stating zaleplon had excellent stability in solution over time. Overall, measuring a drug's specific absorbance by spectrophotometry and comparing to an established literary value is a simple and reliable technique for the quantitative verification of a stock drug solution.

## Acknowledgments

The authors would like to acknowledge Ann Marie Gordon of the San Francisco Office of the Chief Medical Examiner and John Davis of the Orange County Sheriff's Department for providing external zaleplon solutions for verification.

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