Volume2, Issue1, January 2011
Available Online at www.ijppronline.com

International Journal Of Pharma Professional's Research
Research Article

SPECTROPHOTOMETRIC METHOD OF STANDARD CURVE PREPARATION AND CALCULATION FOR METRONIDAZOLE.

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Abstract

Standard curve preparation is the very basic need in field of Formulation or Research with a drug or unknown substance before starting the further calculation for a proposed work. This work is done because most of the students are calculating the values of slope, intercept directly from the software and find manual calculation a tedious job. Also the improper way to handle the glassware and some minor negligence in plotting the graph can differ the results. Many of the students that are first time preparing the standard curve faces many problems basically in searching a correct methodology and has certain queries in regard with the preparation of the standard curve like.

Keywords: - : Standard Curve, Regression, Slope, Intercept, Metronidazole, Calibration curve, UV Spectroscopy,.

Introduction

Preparation of Standard and Regression Curve

Many laboratory tests require the outcome of a carefully controlled chemical reaction be evaluated or read in a photometer (colorimeter or spectrophotometer). Since these instruments are capable of only measuring the amount of light being allowed to pass through the cuvette, their readout devices display % of light transmitted or mathematically derived absorbance. One method of obtaining concentration from % transmittance or absorbance is through the use of a standard curve. For our purposes, standard curves are defined as graphs with absorption or %T plotted on the Y axis, and increasing concentrations of standard along the X axis. If Beer's Law is followed, the resulting line representing absorbance vs. concentration will be straight. A standard curve is constructed after obtaining the %T/Abs readings from a number of solutions of known concentration (standards) used in a reaction or procedure. After the readings are obtained each is plotted on semi-log (% transmittance) or linear (absorbance) paper against the corresponding concentration. If the procedure follows Beer's Law, the points plotted will generally lie such that a straight line can be drawn through them. The concentration of controls and other unknowns (patient samples) can be determined by locating their %T/Abs reading on the line, and then dropping an imaginary line down from that point to intersect the concentration axis. [1]

Linear regression is a statistical technique which is used to estimate or predict the value of one variable, if the value of another correlated variable is known in advance or is assumed hypothetically.

In order to estimate the value of one of the correlated variable from the given value of the other variable, the regression lines are used. [2]

Once the curve is drawn, a number of things must be considered to determine its acceptability. The majority of the curve's points should be on or close to the line. There could be many reasons for a point not being on the line. If the standards are formed from a series of dilutions, the accuracy of the dilutions must be suspect. Calculations of the dilutions and spectrophotometer errors are other possibilities. Whether or not the curve passes through the point of origin (the "0"), varies with the procedure. [1]

The extent of absorption of radiation by a given absorbing system at a specific monochromatic wavelength is governed by two classic laws of absorptiometry.

LAMBERT'S (BOUGNER'S) LAW: - At a given concentration (C) of a homogeneous absorbing system, the intensity of transmitted light decreases exponentially with increase in path light.

BEER'S LAW: - It is concerned with concentration (C) and states that for a layer of defined path length, the intensity of transmitted light decreases exponentially with increase in concentration (C) of a homogeneous absorbing system. If Beer's law is followed and the procedure is linear at the lower concentrations, the curve's line generally goes through the zero.

The combination of both these laws gives the popular law

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i.e. Beer Lambert Law

$$\log Io/I = kcb.$$

Where k= Absorptivity of the system.

When concentration is expressed in g/100 ml., k is described as specific absorption and is designated by the symbol A(1%, 1cm.) i.e. absorbance of a 1%w/v solution of a substance in a cell of 1 cm. path length. [3]

Basic Standard Curve Characteristics

1. Neatness counts. Preparing a good standard curve takes time and practice. A sharp pencil should be used during the early construction period.
2. Use the X axis for concentration. Determining how to space between the individual concentrations is done by trial and error and will also depend on the individual procedure.
3. The Y axis is labelled either %T (semi-log paper) or Absorbance (linear paper). The amount of spacing for absorbance readings is often times determined through trial and error.

The following is an abbreviated list of errors or problems encountered by students in the past. It is provided for you to consider as pitfalls to avoid. You should keep this/these page(s) handy when preparing your chemistry lab curves.

a. Bottom of Y axis did not start at 0.000.
b. Compressed Y or X axises.
c. Uneven spacing of Y or X axises.
d. Not labelling correctly/in the right place.
e. Drawing curve point to point.
f. "Fat" pencil lines/double/smeared lines.
g. Making dots on curve's line for unknown's absorbance value.
h. Drawing dotted lines on graph representing how the concentration of unknown was determined.
i. Drawing circles around dots on the curve line.

The following Standard Curve preparation of the drug Metronidazole will be helpful to figure out the problem regarding procedure.

METHOD:

Solubility of Metronidazole

Reagent: 1N Hydrochloric Acid.

Procedure: Shake the preparation mechanically for 10 min. For uniform making and pipette out quantity of the sample equivalent to 100 mg of the substance into 100 ml. volumetric flask containing 70 ml. of 1N Hydrochloric acid. Rinse the pipette with the acid. Shake it mechanically for 15 min. and make the volume to 100 ml. 1 N acid. Shake well and filter through No.1 filter paper, discard first 10-15 ml. of the filtrate. Dilute 10 ml. of the clear filtrate to 100 ml with 1N acid. [3]

Preparation of Standard Solution: Weigh accurately 100mg of pure Metronidazole & dissolve in minimum quantity of methanol and dilute to 100.0 ml with 0.1 N HCL. Take 10.0 ml of this stock solution & dilute further to 100.0 ml with 0.1 N HCL. Pipette out 0.2, 0.4ml, 0.6ml, …. of this solution & dilute to 10.0 ml in separate 10.0 ml volumetric flask to make 2,4,6,8,..,20.µg/ml concentration solutions. Measure the absorbance (in UV Spectrophotometer) at λ_{max} 277 nm (that has been found by first scanning the sample for *λ max as explained below)*.

λ max Determination (Spectrum Mode in UV Spectrophotometer):

Take the sample of highest concentration for the estimation of λ max from the above prepared aliquots. Fill the reference cell and sample cell (UV Spectrophotometer) with blank i.e 0.1N HCL and then correct the baseline. Then take out the sample cell and fill it with the desired sample and then proceed for scanning. The highest peak will give the λ max for the drug with maximum absorbance.

The λ max was found to be 277nm.

To determine the slope of the line, a graph is plotted between Concentration vs. Absorbance, here the value of slope is directly calculated by the use of MS-Excel.(Fig. No. 1)

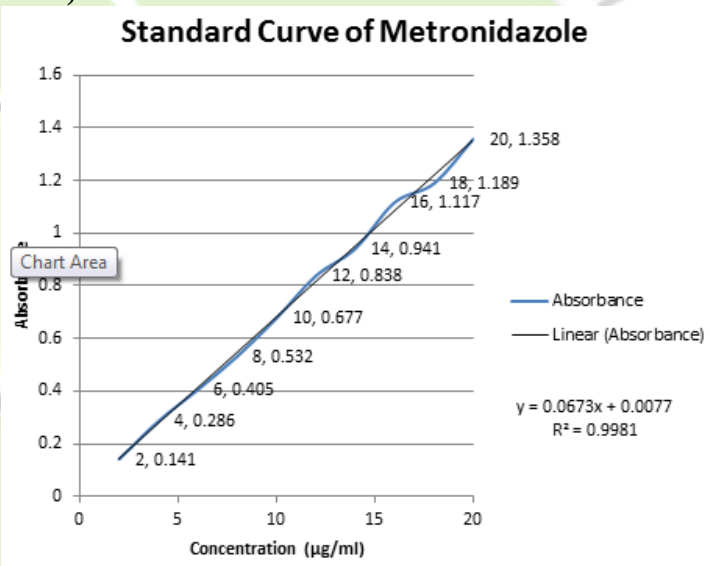

Fig. No. 1. Standard Curve Graph plotted between Concentration and Absorbance.

For the calculation of the slope with given ***two points*** (x_1, y_1) and (x_2, y_2) on a line $\boldsymbol{y = bx + a}$, the slope $\boldsymbol{b}$ of the line is

$$b = \frac{y_2 - y_1}{x_2 - x_1}$$

In mathematics, the slope or gradient of a line describes its steepness, incline, or grade. A higher slope value indicates a steeper incline.

The slope is (in the simplest of terms) the measurement of a line, and is defined as the ratio of the "rise" divided by the "run" between two points on a line, or in other words, the ratio of the altitude change to the horizontal distance between any two points on the line. But in case of

multiple points here is the method to calculate the slope and the value of intercept

Standard curve of Metronidazole (Table. No.1): (For Manual Calculation of the value of slope just follow the given steps)

Equation of regressed line: $y = bx + a$

Where,

$y = Absorbance$

$x = Concentration$

$b = Slope$

$a = intercept$

Slope $b = \frac{n\sum xy - \sum x.\sum y}{n\sum x^2 - \sum(x)^2}$ & **Intercept** $a = \overline{y} - b\,\overline{x}$

- ***Just after getting the result Table.No.2 was prepared for ease in the further calculations.***

Table.No.1. Standard Curve Table (Readings of absorbance against the prepared concentrations obtained from UV Spectrophotometer Fig. No.3)

S.No	Concentration	Absorbance
1	2µg/ml	0.141
2	4 µg/ml	0.286
3	6 µg/ml	0.405
4	8 µg/ml	0.532
5	10 µg/ml	0.677
6	12 µg/ml	0.838
7	14 µg/ml	0.941
8	16 µg/ml	1.117
9	18 µg/ml	1.189
10	20 µg/ml	1.358

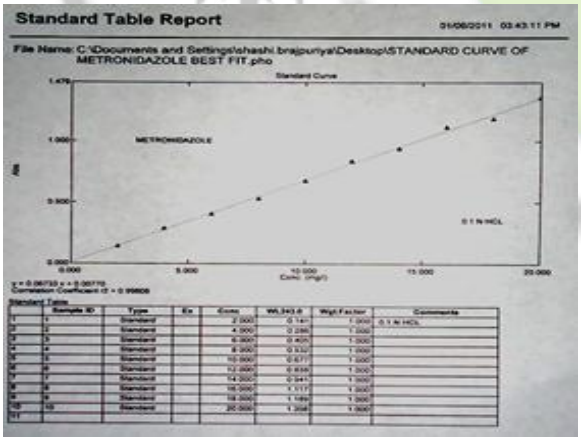

Fig. No. 3. Reading of the absorbance obtained from UV Spectroscopy Schimadzu 1800.

STEPS FOR CALCULATION [4]

Step 1: Calculation of Slope i.e. the value of 'b'.

By the formula:

$$\mathbf{b} = \frac{n\sum xy - \sum x.\sum y}{n\sum x^2 - \sum(x)^2} \quad \text{..... (1)}$$

Where,

n= no. of observation i.e. 10

$\sum xy$= 104.546

$\sum x$= 110

$\sum y$= 7.484

$\sum x^2$= 1540

$(\sum x)^2$ = 12100

Table. No. 2. Observation charts for the calculation of slope and intercept.

S.NO	Concentrations	Absorbance		
	x	y	x^2	xy
1	2	0.141	4	0.282
2	4	0.286	16	1.144
3	6	0.405	36	2.43
4	8	0.532	64	4.256
5	10	0.677	100	6.77
6	12	0.838	144	10.056
7	14	0.941	196	13.174
8	16	1.117	256	17.872
9	18	1.189	324	21.402
10	20	1.358	400	27.16
∑	**110**	**7.484**	**1540**	**104.546**
n	**10**	**10**	**10**	**10**

- *Values are obtained from Table.No.2*

By putting these values in equation [1], we get

$$b = \frac{10*104.546 - 110*7.484}{10*1540 - 12100}$$

$$b = \frac{1045.46 - 823.24}{3300}$$

$$b = \frac{222.22}{3300}$$

Therefore, **b = 0.06733**

Step 2: Calculation of Intercept i.e. the value of 'a'.

$$a = \overline{y} - b\,\overline{x} \quad \text{..... (2)}$$

Where,

$\overline{y}$ = mean of y. (sum of observations/ no. of observations).

$\overline{x}$ = mean of x. (sum of observations/ no. of observations).

b = slope.

$\overline{y}$ =∑y/10 $\overline{x}$ =∑x/10

$\overline{y}$ = 7.484/10 $\overline{x}$ = 110/10

$\overline{y}$ = 0.7484 $\overline{x}$ = 11

By putting the values of $\overline{y}$ and $\overline{x}$ in equation 2, we get

i.e. $a = \overline{y} - b\,\overline{x}$

a = 0.7484 - 0.06733*11

a = 0.7484 – 0.74063

a = 0.00777

Step 3: Calculation of regressed values (Table. No.3) (that means how much the values of y are regressed) for 'y-$_1$', 'y$_2$', 'y$_3$'……. by putting the values of 'x1', 'x$_2$', 'x$_3$'……. respectively along with 'a' and 'b' in the equation of line will give the regressed values of y.

i.e. $y = bx + a$

Now for calculating the values for regressed y, we can use the same equation of line for which the variables will be as follows:

i.e. $y = bx + a$

Where, y = absorbance.

x = concentrations.

b = Slope.

a = intercept.

Table. No.3. Table For the calculation of Regressed values of y (y_1 to y_{10}).

S.No.	Slope X Conc. (bx)	Intercept (a)	Regressed Values ($y = bx + a$)
1	0.06733*2 (x_1) = **0.134678788**	0.0077	0.134678788 + 0.0077 = **0.14234545** (y_1)
2	0.06733*4 (x_2) = **0.269357576**	0.0077	0.269357576 + 0.0077 = **0.27702424** (y_2)
3	0.06733*6 (x_3) = **0.404036364**	0.0077	0.404036364 + 0.0077 = **0.41170303** (y_3)
4	0.06733*8 (x_4) = **0.538715152**	0.0077	0.538715152 + 0.0077 = **0.54638182** (y_4)
5	0.06733*10 (x_5) = **0.673393939**	0.0077	0.673393939 + 0.0077 = **0.68106061** (y_5)
6	0.06733*12 (x_6) = **0.808072727**	0.0077	0.808072727 + 0.0077 = **0.81573939** (y_6)
7	0.06733*14 (x_7) = **0.94275152**	0.0077	0.94275152 + 0.0077 = **0.95041818** (y_7)
8	0.06733*16 (x_8) = **1.077430303**	0.0077	1.077430303 + 0.0077 = **1.08509697** (y_8)
9	0.06733*18 (x_9) = **1.212109091**	0.0077	1.212109091 + 0.0077 = **1.21977576** (y_9)
10	0.06733*20 (x_{10}) = **1.346787879**	0.0077	1.346787879 + 0.0077 = **1.35445455** (y_{10})

If a graph is plotted between the Regressed values obtained from Table.no.3 and the Absorbance it will be the line of regression and the curve will be the Standard Regressed Curve (Fig. No.2). Here the value of R^2 (Regression Coefficient will be equal to 1, that means the line is perfect straight.)

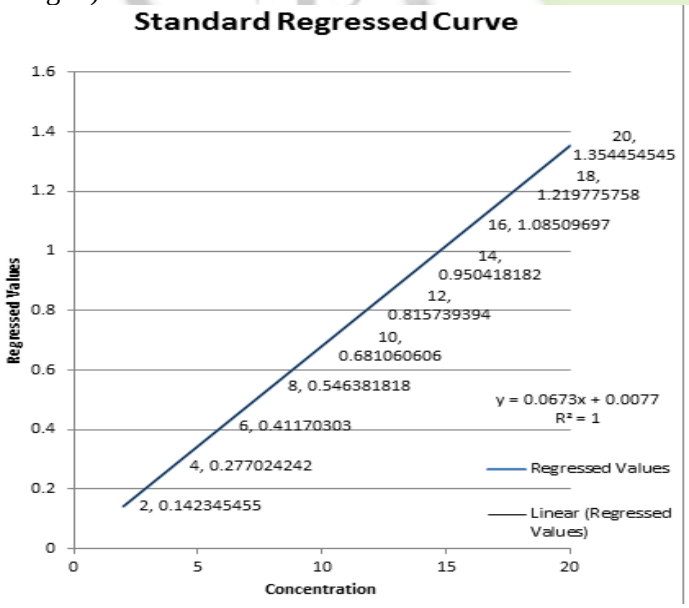

Fig.No.2. Standard Regression Curve Graph plotted between Concentration and Regressed values.

Result And Discussion

Standard calibration curve of Metronidazole was prepared in the study and a procedure in detail was explained so that it should be ease for the beginners who are going to prepare the standard curve the very first time. In the present study standard curve is prepared with the slope of line as 0.067, intercept as 0.007 and regression coefficient as 0.998 for the same the values of regressed points of y had also calculated and the graph is plotted for the same with regression coefficient as 1. The above work is done because most of the students are calculating the values of slope intercept directly from the software and find manual calculation a tedious job. Also the improper way to handle the glassware and some minor negligence in plotting the graph can differ the results.

References

1. http://www.austincc.edu/mlt/chem/chemlab3cstandardcurve.pdf
2. Dhall, G.D.; Chhibber, S.N.; Trivedi, Hari Om; Chandra, Subodh; Frank mathematics for B. Pharm, First Edition 2004, Frank Bros & Co. (Publishers) Ltd., Pg. No. 13.25.
3. Sethi, P.D.; Quantitative Analysis of Drugs In Pharmaceutical Formulations, Third Edition, CBS Publishers & Distributors, New Delhi, Pg. No. 18, 226.
4. Vogel, Arthur Israel; Vogel's textbook of Quantitative Chemical Analysis, Fifth Edition, Longman Group UK Limited, Pg.No. 144-145