

# Accurately Precise and Precisely Accurate: An Experimental Comprehension

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**Abstract** Accuracy and precision are the primary targets in chemical analysis. This article is an experimental approach to appreciate the concepts of precision and accuracy in analytical chemistry. An easy, fast and well-established spectrophotometry experiment for quantitative analysis of  $\text{Fe}^{2+}$  is done using 1,10-phenanthroline as a complexing or coloring agent. Real time learning approach is carried out; it means discussing the data in front of class after collection of experimental results. A little data is used to discuss variety of concepts of basic statistics in analytical chemistry. A debate regarding priority of precision over accuracy is done. The objective of using standard deviation (STDEV) and changes in its value by unit conversion or other transformation is explained. The advantage of using relative standard deviation (RSD) is also demonstrated. Several spectrophotometers are compared regarding precision. By using same data, precision of analog and digital spectrophotometers is also compared. Repeatability and reproducibility are also explained and calculated using the same data. Presence of systematic error or accuracy of experiment is statistically assessed without doing further experimentation. The whole study is optionally supported with online MS-Excel worksheets that are automated for better understanding.

**Keywords:** accuracy, precision, second year undergraduate, analytical chemistry, graduate education/research

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## 1. Introduction

Statistics is an essential tool for quality analytical chemistry [1]. The prime target of using statistics is to have accurate and precise outcomes of analysis. Stories about accuracy and precision are not new, people are always worried about it [2,3]. This is well-known concept that closeness of data is precision and closeness of results or its mean value to the true or actual value is accuracy. Several attempts have been made in past to nourish the concept of accuracy and precision in the field of chemical education (4). Experiments and pedagogical contents have been reported for good understanding of the concept of basic statistics, especially accuracy and precision [5,6]. Other chemical analysis [7,8] as well as disciplines covering other than science subjects are also emphasis on accuracy-precision differentiation even in topic of articles [9,10,11].

### 1.1. Real Time Teaching and Learning

This is an approach to discuss the concepts within lab or during experiment. In this experiment, a collective data is used and students are gathered to discuss data and do calculation to appreciate concepts. At each step students are asked reason(s) for any expected and unexpected matter, details are demonstrated in discussion section.

This gives chance to students to actively think and practice their concepts. Most of the concepts canvassed in this article are commonly known, but it is always better to practically appreciate them.

### 1.2. Which Comes First? Accuracy or Precision

An important thing that is discussed (prior to experiment) is preference among accuracy and precision. Both are important but which one has priority, it has to be understood. Usually people have different misconceptions in treatment of data [12]. One of those is about giving preference to accuracy or precision. A website has been visited in August 2011 and also cited in this article [13] showing a poll for choosing more important among accuracy and precision. In such poll out of 22 participants, 12 gave preference to accuracy over precision. This is not necessary to have same results of such polls every time but usually the students of chemistry or analytical chemistry also give importance to accuracy over precision. That argument is exercised in current study. Details are specified in subsequent text (see section 4.1.).

### 1.3. Why Standard Deviation?

Since precision is the closeness to the mean value of data, therefore deviation (i.e. difference of each data point with mean) can be one of the tools to know closeness of

data. But problem is that approximately half of the deviation values are always negative with similar magnitude as of positive values. In order to find out the average of deviation one should always find zero value of average deviation or value very near to zero. Usually for averaging the data having both positive and negative values, a root mean square is calculated. Therefore square of deviation turns negative values to positive and then after calculating their average, square root of resultant is calculated. This value is termed as standard deviation (STDEV) in terms of statistics. That is basically root mean square value of deviation (equation 1) and holds good identity for precision of data.

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad (1)$$

#### 1.4. Transformations Can Change Standard Deviation

STDEV has the same unit as of each data point. If value of each data point is transformed or manipulated then the value of STDEV is also changed as well as its unit. This fact is also explained and practiced in this study. There is a way to normalize such transformation that having a value showing precision but does not change with transformation or manipulation. This is relative standard deviation (RSD) that is the ratio of STDEV with mean value of same data. RSD is a well-known statistical terminology and for all type of transformations it usually remains same. This is also well practiced and evidenced in this experiment.

#### 1.5. Comparisons Regarding Precision

In current study precision of spectrophotometers is compared individually and also by categorizing them in analog and digital equipment. Comparisons of precision on different basis or categories (like among students, gender etc.) are also possible in this experiment but not considered in this study. Criterion for precision comparison is the value of STDEV. Lesser the value of STDEV means better precision.

#### 1.6. Repeatability and Reproducibility

These are also useful statistical terminologies; repeatability refers to the precision of replicate analysis while reproducibility refers to the precision between different sets of analysis. For repeatability, all experimental conditions for each replicate are considered to be identical and also all runs are at same slot of time. In case of reproducibility there must be difference in one or more of the conditions like equipment, time or instance, analyst, method etc. Within-run precision is repeatability and between-run precision is reproducibility. These are also estimated in this experiment to practice such concepts [14].

#### 1.7. Regarding Accuracy

Accuracy is related to the systematic errors in the data. Statistical confidence level is good for identifying systematic error in analysis. Important is that the true value ' $\mu$ ' of analyte in sample must be known. Number of

replicates ' $n$ ', their mean ' $\bar{x}$ ' and STDEV ' $s$ ' are used to find out confidence interval (equation 2) corresponding to value ' $t$ ' that is tabulated statistically [14].

$$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \quad (2)$$

In case that true value lies in the confidence interval (equation 2) of  $x + \frac{ts}{\sqrt{n}}$  and  $x - \frac{ts}{\sqrt{n}}$ . One can state that at the certain level of confidence (for which ' $t$ ' is tabulated) there is no systematic error in data or analysis.

### 1.8. Brief of Chemistry in Analysis

A well-established and simple spectrophotometric determination of  $\text{Fe}^{2+}$  is carried out in this study [15]. Complexing agent (1,10-phenanthroline) forms fairly colored complex with  $\text{Fe}^{2+}$ . This complex shows highest absorbance approximately at 510nm. Complex is stable under a certain pH range. Acetic acid/ Sodium acetate buffer is used, with support of hydroxylamine solution as reducing agent in order to avoid unnecessary oxidation of  $\text{Fe}^{2+}$ . Although this study can also be done far easier and quicker way, by analyzing readily colored analytes (like  $\text{CuSO}_4$ ,  $\text{KMnO}_4$  solution etc.).

## 2. Materials and Methods

### 2.1. Pre-lab

The scope, objectives, experimental details and hazards related to this study are instructed to students before experiment. Priority among precision and accuracy is also discussed before experiment.

### 2.2. Preparations by Instructor

Distilled water is used throughout the analysis. A stock solution of 20ppm  $\text{Fe}^{2+}$  is prepared from analytical grade ammonium iron (II) sulfate hexahydrate in 500mL volumetric flask as a collective sample. This stock sample solution is acidified with 5mL of 98% sulfuric acid before dilution and was made available to student in a good quality 50mL graduated burette. In order to avoid any biased result, concentration of this stock sample is not given to students. This concentration is informed to students after all experimental outcomes are entered in Table 1 at classroom's board.

Approximately 100 g of hydroxylamine hydrochloride is dissolved and diluted up to 1 liter with distilled water. Sodium acetate solution is prepared by dissolving ~200g anhydrous sodium acetate in 1 liter of distilled water. 2.5g of 1, 10-phenanthroline monohydrate is dissolved in 1 liter of ethanol.

### 2.3. Preparations by Students

Students are instructed to dilute (1:10) the above stock sample in three 25mL volumetric flasks (for triplicate analysis) with addition of hydroxylamine hydrochloride, sodium acetate and 1,10-phenanthroline solutions (2.5mL of each) before making up of volume.

Each group of students is instructed to make appropriate stock  $\text{Fe}^{2+}$  calibration standard solution and

acidify accordingly with sulfuric acid (as mentioned earlier in preparation of stock sample solution). One working calibration standard of  $\text{Fe}^{2+}$  is diluted within the range of 0.02 to 0.06mM from stock  $\text{Fe}^{2+}$  calibration standard. 2.5mL of each of hydroxylamine hydrochloride, sodium acetate and 1,10-phenanthroline solutions are also

added prior to making up of volume of working calibration standard. Students of group '1' choose 0.04mM  $\text{Fe}^{2+}$  as working calibration standard (Table 3). Blank solution is prepared by diluting 2.5mL of hydroxylamine hydrochloride, sodium acetate and 1, 10-phenanthroline solutions in 25mL volumetric flask.

**Table 1. Consolidated Data of Entire Class and Outcomes of Calculation. (Portion in gray background is reproduced from classroom board after experiment)**

Instrument code	Instrument type	Group Number	Average			STDEV			RSD		
			Absorbance	Concentration (mM)	Concentration (ppm)	Absorbance	Concentration (mM)	Concentration (ppm)	Absorbance	For mM Concentration	For ppm Concentration
A	Analog	1	0.481	0.033	1.843	0.05147	0.00635	0.35469	0.12561	0.16295	0.16295
		2	0.399	0.045	2.513						
		3	0.401	0.044	2.452						
		4	0.358	0.034	1.899						
B	Analog	5	0.363	0.045	2.519	0.01953	0.00702	0.39213	0.05468	0.19383	0.19383
		6	0.335	0.039	2.150						
		7	0.381	0.030	1.659						
		8	0.350	0.032	1.765						
C	Analog	9	0.483	0.028	1.580	0.05502	0.00573	0.31979	0.13503	0.17936	0.17936
		10	0.373	0.034	1.871						
		11	0.361	0.039	2.195						
		12	0.413	0.027	1.486						
D	Digital	13	0.385	0.036	2.010	0.01307	0.00275	0.15379	0.03363	0.07295	0.07295
		14	0.401	0.035	1.955						
		15	0.397	0.041	2.290						
		16	0.372	0.039	2.178						
E	Digital	17	0.365	0.029	1.620	0.05221	0.00507	0.28293	0.13380	0.15588	0.15588
		18	0.337	0.034	1.899						
		19	0.401	0.039	2.178						
		20	0.458	0.028	1.564						
F	Digital	21	0.354	0.029	1.620	0.02704	0.00716	0.40013	0.07061	0.19364	0.19364
		22	0.367	0.033	1.843						
		23	0.399	0.042	2.346						
		24	0.412	0.044	2.457						
<b>Column Statistics</b>		Average	0.389	0.036	1.994						
		STDEV	0.0397	0.0058	0.3266						
		RSD	0.1021	0.1637	0.1638						

## 2.4. Data Acquisition

Six spectrophotometers of two types are employed. Three Erma<sup>®</sup> AE-200 analog and three Thermo<sup>®</sup> Genesys-6 digital spectrophotometers are used. All instruments are assigned codes as mentioned in Table 1. Students are distributed into 24 groups and four groups are assigned to do their experiment at each instrument (Table 1). Absorbance measurements are carried out at 510nm in 10mm optical grade plastic cells against blank solution. Portion of Table 1 with gray background is made at the classroom board. Each group of students entered the data in respective rows at board after experiment.

## 3. Hazards

No extremely hazardous substance is used in this experiment. Care should be taken to avoid skin contact and wear eye protection. Acidification of stock  $\text{Fe}^{2+}$  solution must be carried out in well-ventilated area preferably in fume hood.

## 4. Discussion

This experiment is done by 72 students. They were divided in 24 groups such that each group has three

students. This experiment is repeated in six semesters in three years. Around 150 students have participated all-over. Currently, experiment that has done in one semester is reported only. This is a collective experiment therefore whole class did most of the calculations altogether. For some calculations, only data or results of group 1 are given as an example. MS Excel™ worksheets as online support for this study are available online at <http://1drv.ms/1LFKvYp>. See details in section of 'Available Supporting Information' at the end of this article. This article is also understandable without such support. In this case ignore brackets [ ] in following text.

#### 4.1. Accuracy Vs. Precision

First of all a question is asked to students about preference between accuracy and precision. Approximately 70% of students were in favor of accuracy, 25% with precision and 5% had opinion that both have equal importance. This is further discussed with the help of examples in which this is asked that if outcomes of a titration X are 2.9, 17.1, 8.9, 11.1mL with average of 10.0mL. In another similar titration Y having endpoints 9.5, 9.6, 9.5, 9.4mL with average of 9.5mL. Which titration is looking fine? Almost all replied that Y is looking good. The only reason is good precision. If the true or real value of end point is given (e.g. 10.0mL) then it seems that X is better regarding accuracy. Since average of analysis matches the true value. But still most of the students choose Y again just because of precision. Another important fact is that most of the time the true value for samples is not known in real quantitative analysis. Therefore mostly there is no idea about accuracy. But precision is always there, unless you don't have replicate analysis. Fortunately they all agreed that precision has more importance.

#### 4.2. Real-time Learning

The better way of learning is to call all the students at classroom's board, after students have finished their experiment and entered outcomes at board. The portion of the Table 1 having gray background is reproduced from the classroom board. This approach of real-time learning is beneficial for students as this is a good exercise of their concepts. In preceding text, it has already been discussed that precision has priority over accuracy regarding importance. Therefore precision is assessed before accuracy in the remaining section. Further aspects as real-time teaching and learning are continued in subsequent text.

#### 4.3. Difference in the Values of Standard Deviation

Prior to do any calculations, students are asked that what they expect about STDEV values of columns (Absorbance, concentration in mM and ppm). Whether they will be same or not? This question creates groups in students having opinion yes and no.

Then students are asked to calculate the STDEV (using equation 1) for first two columns (Absorbance and concentration in mM units). The values of STDEV are different, i.e. 0.0397 [Table 1/E33] and 0.0058 [Table 1/F33] (Table 1) for absorbance and concentration (in mM), respectively. The reason for this difference is asked

to the students. This is because these are the STDEV of different values therefore these are not same. Then it is also asked that what about the unit of STDEV? Answer is that the STDEV has the unit same as each individual data has. For example, unit for STDEV of molar concentration values is molarity.

Then it is asked to students that how they can estimate the STDEV for concentration (mM) from the value of STDEV of Absorbance. It means as they have calculated the value for concentration (mM) using single standard calibration for their sample (equation (3), where  $A$  is absorbance,  $C$  is for concentration; subscript  $u$  and  $k$  represents unknown and known concentrations).

$$\frac{A_u}{A_k} = \frac{C_u}{C_k} \quad (3)$$

Similarly they have to estimate the value for STDEV of concentration (mM) by placing the value of STDEV of sample's absorbance 0.0397 (from column statistics of Table 1) in place of average absorbance  $A_u$ . Therefore the equation 3 is transformed to the following equation (4), where 's' is STDEV.

$$\frac{s_u^A}{A_k} = \frac{s_u^C}{C_k} \quad (4)$$

The result of this calculation (using equation 4) is an estimated STDEV for concentration (mM) that is 0.0027mM. Absorbance and concentration of working standard are used from data obtained by group 1 from Table 3. This value (0.0027mM) is somehow closer to the STDEV obtained directly from the column having concentrations (mM) in column statistics of Table 1 (i.e. 0.0058mM) [Table 1/F32]. The reason for having difference in these values is very interesting. Since each group uses different calibration standard solutions for such estimation. Hence there must be different degree of errors in preparation and measurement of calibration standards by each group. Therefore values of estimated STDEV and directly calculated STDEV are different.

Similarly students are asked to estimate STDEV for concentration in ppm using STDEV of concentration (mM). They used the conversion factor as they have used to convert the mean concentration (mM) to ppm. In this case, the conversion factor is 55.847. Therefore, the estimated value for STDEV of concentration (in ppm) is 0.3240 that it very close to direct STDEV calculation of column in Table 1 (i.e. 0.3266) [Table 1/G33]. Unlike conversion from absorbance to mM concentration, both concentration columns have similar conversion factor for each observation. Therefore the estimated and directly calculated STDEV values are closer. Sometimes a little difference in these values may appear. This may be due to the difference in selection of significant figures in calculation by different groups.

#### 4.4. How relative Standard Deviation is Advantageous?

RSD is the ratio of the STDEV and mean of replicates. Therefore it normalizes the deviation effect. RSD values for absorbance and both kind of concentration values are expected to be same. But there are interesting facts in column statistics of Table 1 that RSD for absorbance is

different from RSD of corresponding concentration values. But RSD values for both concentration columns are same. This is again discussed with students that the absorbance does not have effect of calibration standard. Since different calibration standards are used in groups hence different calibration factors are used for each data point. Therefore the RSD values are different. In case of both concentration units, these are same because for each observation a fixed conversion factor is used. Unlike collective data in Table 1, in Table 3 the data is from single group [Table 3'E19 to G19]. The RSD values are same for all columns. Since single group uses standard of fixed concentration of working standard, it means same calibration factor is employed for all replicates. Most of the times RSD does not change with conversions hence this seems to be a better and more comparable tool to estimate precision than STDEV.

#### 4.5. Comparing Equipment

Data is organized in categories of groups that have used same equipment. Then precision for each instrument is calculated. STDEV for absorbance and concentration in mM and ppm units are mentioned in Table 1 under the column heading of STDEV. Regarding instrument's performance it is better to do comparison of absorbance instead of concentrations. In case of concentration columns, the use of calibration standards compensates the errors. So, one could not perfectly find the precision profile of standalone equipment. The lowest of STDEV value for absorbance 0.01307 [Table 1'H19] or highest precision is of instrument 'D' that is digital equipment. The poorest in precision is equipment 'C' (i.e. analog) with STDEV for absorbance value 0.05502 [Table 1'H15].

Interesting thing is that if the STDEV for concentration in mM or ppm from each instrument is compared then still the best is 'D' but the worst is now 'F' (digital) [Table 1'I27]. Again the fact behind this observation is

that the ratio of absorbance values in calculation of concentration (using equation 2) compensates the errors. Therefore it is always recommended in analysis, to run fresh calibration standard along with the sample.

#### 4.6. Analog vs. Digital

Out of which first three (coded: A, B and C) are analog and others are digital. According to Table 2, precision for analog equipment is less or STDEV (0.0479) [Table 2'D6] is higher than digital equipment (0.0316) [Table 2'D7]. This may be due to the manual reading of absorbance displayer at analog spectrometers (i.e. a galvanometric needle). Therefore chance of uncertainty is higher. Others (D, E, and F) are having digital displayer that has less chance of human error in observing absorbance values. Also several techniques for signal to noise ratio improvement are being used in modern or digital equipment [16].

Table 2. Precision Comparison for Analog and Digital Equipment.

Instrument Type	STDEV For Absorbance
Analog	0.0479
Digital	0.0316

#### 4.7. Repeatability vs. Reproducibility

It has been mentioned in previous text that within-run precision is repeatability and between-run precision is reproducibility. Students are asked to identify the cases of within and between runs. Data in Table 3 is observed by single group and also represents within-run system. Since six spectrophotometers are used in this experiment Therefore one can estimate repeatability that is STDEV for the replicates run by each group. For group 1 the repeatability (i.e. STDEV) in absorbance measurement is 0.0030 [Table 3'E18].

Table 3. Experimental Outcomes for Single Group of Students.

Group Number	1		
Instrument Type	Analog		
Instrument Code	A		
Concentration Iron(II) working standard (mM)=	0.04		
Absorbance for Standard Ferrous solution =	0.583		
<b>Absorbance for Sample solution:</b>			
Replicate	Absorbance at 510nm	Concentration (mM)	Concentration (ppm)
1	0.481	0.0330	1.843
2	0.478	0.0328	1.832
3	0.484	0.0332	1.855
<b>Column statistics:</b>			
Average	0.481	0.0330	1.8430
STDEV	0.0030	0.0002	0.0115
RSD	0.0062	0.0062	0.0062

For estimation of reproducibility, conditions between the runs must be changed. This means there must be difference in at least one of the analysis parameters like date of analysis, analyst, temperature, equipment etc. for single sample. In this experiment different instruments and groups of analysts are involved in analysis of the same

sample. Therefore the STDEV for absorbance measurements by all the groups is 0.0397 [Table 1'E33] and this can be referred as reproducibility of analysis (Table 1). The values are according to expectations that repeatability is improved in comparison with reproducibility. STDEV for same equipment is usually

lesser than measurements using different equipment. One can also estimate repeatability and reproducibility values for concentration (mM) and in ppm.

#### 4.8. Accuracy Reconciliation

After experiment, students are informed about the actual concentration of the collective sample they have analyzed (i.e. 20.0ppm Fe<sup>2+</sup>). Since this sample is diluted 10 times (see section 2.3.) therefore it became 2.0ppm Fe<sup>2+</sup> after dilution. This is true value ' $\mu$ ' as described in equation 1. The mean ' $\bar{x}$ ' concentration value (in ppm) for triplicate runs is 1.8430 [*Table 3'!G17*] and STDEV i.e. 's' is 0.0115 [*Table 3'!G18*] from *Table 3*. Statistical value of 't' is tabulated 4.303 at two tailed basis with confidence level ' $\alpha$ ' = 0.05 for 2 degree of freedom. The

calculated value of term  $\frac{ts}{\sqrt{n}}$  in equation 2 is 0.0286.

Finally, using equation 2 the estimated range for ' $\mu$ ' is found 1.872 to 1.814. Since true value ( $\mu = 2.0$ ppm) does not lie in this range. It can be stated statistically that at 95% confidence level there is a systematic error in the analysis performed by group 1. In case if true value lies in the estimated range then one can state that there is no systematic error in the analysis at 95% confidence level. The above range can also be calculated for other confidence levels. One of the students asked the reason about not using absorbance data for assessment of accuracy. The reason is that it is difficult or quite impossible to have true value for the absorbance measurement in this case.

#### 5. Conclusion

This activity is based on the good understanding of basic statistical tools used in the field of analytical chemistry. The experiment is simple, easy and fast. Small amount of less harmful chemicals are used. Usual spectrophotometers are used that are mostly available in academic and other laboratories. Experiment can hold large number of students as it requires less space and gives chance to each student with active participation. This is collective lab for whole class. A real-time approach is used to discuss the collective and individual experimental outcomes by sharing ideas with students. A little data is used to appreciate variety of concepts.

#### 6. Available Supporting Information

MS-Excel™-2003 worksheets are available online at <http://1drv.ms/1LFKvYp> (file named *Support File(1).xls*) to support understanding of tables and also confirm calculations in this article. This file can be used to do calculation for your own experimental data. By reason the cells in the worksheets are not locked. Therefore it is recommended to enter the values only in the cells having gray background. This will automatically do calculation in rest of the cells having formula. Reference of the cells in worksheets are given in italics within the square bracket [*'Sheet's name'! Cell address*] in the manuscript. Those who are not using this software support may ignore such brackets. This MS Excel software support and other MS

Word document files (Instructor's\_Instructions[1].docx and Handouts\_for\_students[1].docx) are also available via Internet website <http://1drv.ms/1LFKvYp>. All these files can be downloaded and also edited online at internet browser.

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#### List of Abbreviations

STDEV	Standard Deviation
RSD	Relative Standard Deviation.

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