

INTRODUCTION TO GOOD LABORATORY PRACTICES (GLPs)

The following notes on proper experimental technique and use of equipment are collectively known as GLP's. It is assumed that the student is familiar with and always practices the following procedures outlined in this section. Your laboratory instructor will be evaluating your experimental technique. Failure to practice the following will lead to poor results and poor technique. Both of these are graded. Exceptions to standard GLP's must be noted in your lab report.

1. Cleaning and Storing Glassware

Care must be taken to ensure that glassware is thoroughly clean before use. However, recognize that soap can be a serious chemical contaminant. Do not clean your glassware with soap unless specifically instructed. In general, glassware will be supplied clean, acid washed and thoroughly rinsed with deionized water. Properly cleaned glassware is indicated by the presence of an unbroken film of water on the surface. It is seldom necessary to dry glassware before use; in fact this practice should be discouraged because it wastes time, can be a cause of contamination and result in changes in volumetric glassware.

Always pre-rinse burets and volumetric pipettes with the titrant or solution to be transferred prior to use. Note that rinsing is most effectively accomplished with a greater number of small portions, rather than a smaller number of large portions.

Rinse all glassware with tap and distilled water after use. **Never let reagents dry in volumetric glassware.**

2. Housekeeping

Good housekeeping is important for the **safety** and convenience of everyone, including the cleaning staff, who are not chemists. The analytical lab is a shared space. It is a busy place, and any mess you don't clean up will inconvenience many people. A mess will not impress your lab instructor and you certainly won't impress any future employers with poor, unsafe work habits.

Don't leave equipment and chemicals scattered over the benchtop; return them when you are finished using them. This allows others to use the same equipment, and prevents any accidents involving or resulting from your mess. Fumehood space is often at a premium, so clear out as soon as you no longer need to work there. When you are finished working, in the fumehood especially, clean the entire area with a damp paper towel. Clean up all chemical spills immediately, especially when the balances or other instruments are involved. Wash the outside of reagent bottles when you are finished using them. Drips and small spills may go unnoticed until they have had some time to react and cause a burn, at which point it is too late.

Never leave experiments unattended. If you must, especially in the case of reactions and digestions or anything that involves the use of hotplates and stirrers, inform your lab instructor

and have them or another student keep an eye on your experiment. Even if you have to leave the lab for only a minute to go to the bathroom, inform your lab instructor.

When using chemicals and supplies, use all of one container before opening another one, unless specifically directed to do otherwise by your lab instructor. Inform your lab instructor of any chemicals or supplies that are running low, including cylinders of compressed gases and recorder/plotter supplies, so that there will be enough for the next student to complete their experiment. Also inform your lab instructor as soon as possible if anything is missing or if anything is broken, so that replacements can be obtained as quickly as possible.

Dispose of all refuse in the appropriate waste container as soon as possible; if in doubt ask your lab instructor. Questions are not 'stupid'; unlike endangering the lives and health of others. At the end of the lab, return all chemicals and equipment where they belong and clean up your work area. Double-check everywhere you worked, make sure that all equipment and supplies are returned, and all waste appropriately disposed (including any bench and fumehood space, the balances, instruments and sinks where you worked). As far as possible, shut down and turn off all equipment that you have worked with as soon as you have finished.

3. Use of Tap, Distilled and Deionized Water

There are three grades of water available for use in the lab:

- tap
- distilled
- deionized

The three grades of water are progressively more expensive to produce. In general, *use only the minimum purity of water necessary* and do not waste water, particularly the more expensive grades. Distilled water will suffice for most uses.

4. Handling Reagents and Solutions

Successful analytical work depends on the purity and quality of the available reagents. A freshly opened reagent container can be used with confidence; whether or not the stated assay values for purity and impurities remain valid depends entirely on how the container has been handled since being opened. The purity of the chemical reagents available (and the quality of your results and, therefore, your marks) depends on strict adherence to the following rules.

Use small pre-cleaned beakers (in 10 and 20 mL sizes) for pouring out reagents. Any solid or liquid not used should be disposed of appropriately.

- 1 The possibility of contamination can be minimized by choosing the smallest bottle that will supply the required quantity of reagent. Ensure that the purity of the reagent is sufficient to prevent contamination and interferences and to reduce the blank to a minimum. In general, try to use only reagents whose purity you can be certain of (check assay values on the label).
- 2 Replace the top of the reagent container immediately after removal of the reagent.
- 3 Hold stoppers between the fingers; stoppers should never be set down on the bench or anywhere else except in the neck of the appropriate flask or reagent bottle.
- 4 Unless specifically directed to the contrary, never return any excess reagent or solution to a reagent bottle. Contamination of the entire bottle by returning excess reagent is a false economy - considerable time can be spent determining the source of any contamination (and consequent poor results), and the entire bottle then has to be disposed of and a new bottle obtained.
- 5 Before taking a bottle of chemical to the weighing room, first clean your spatula with de-ionized water. Scrub it dry with a Kimwipe. Now take your solid chemical to the weighing room and weigh it.
- 6 Keep reagent storage areas and the balances clean. Clean up any spilled chemicals immediately.
- 7 Do not use a new or unopened bottle of reagent without first obtaining the permission of the lab instructor.

5. Handling Solids

5.1 Balances

Both analytical and top-loading balances are available. Use the top-loading balances wherever possible, i.e., whenever the weight does not have to be exact or where an uncertainty of 0.01 g will suffice. For all work where the weight has to be known as exactly as possible, use the analytical balances.

Electronic Balances:

Most of you will have used the self-taring electronic balances in other courses. In general:

- keep the balance pan and surrounding areas clean
- weigh solids into a container that is as light as possible
- an initial tare weight is usually unneeded, since you can tare the container to zero.
- CLEAN UP when you are done

Detail: weighing solids

Ensure that the solid is of fairly uniform texture and will readily pour. If necessary, shake the capped reagent bottle and/or tap it on a wooden surface to break up any large lumps to allow the reagent to pour freely. Remove the cap and pour a slight excess into a clean, dry beaker or weighing boat. Immediately replace the cap on the reagent bottle and tighten it. Weigh reagent from the beaker or weighing boat into the desired container(s). If necessary, obtain additional solid from the reagent bottle as described above. When finished, return the sealed reagent bottle and properly dispose of the excess reagent.

5.2 Quantitative Transfers

After weighing a solid, it usually must be transferred in its entirety to a volumetric flask or beaker. This process is referred to as a quantitative transfer.

- Take your notebook, washbottle, funnel and receiving vessel (beaker or flask) into the weighing room.
- After weighing your solid and recording the mass, rinse it from the weigh boat through a funnel into the receiving flask.
- Make sure all of the solid has been rinsed from the weigh boat and the funnel into the receiving flask.
- Remove the funnel while rinsing the stem into the flask.

5.3 Ovens and Desiccators

Most solids absorb atmospheric moisture and, as a consequence, change in composition. This effect can be substantial when a large surface area is exposed to a humid atmosphere, as with a reagent that is a fine powder. It is ordinarily necessary to dry such solids before weighing to free the results from dependence upon the atmospheric humidity. Oven drying is the most convenient method for removing absorbed moisture from a solid. This technique, of course, is not appropriate for samples that change composition at the temperature of the oven. Furthermore, with some solids the temperatures attainable in ordinary drying ovens are insufficient to completely remove bound water.

While cooling, dried material is stored in a desiccator to prevent the uptake of atmospheric moisture. The base of the desiccator contains a chemical drying agent. Samples are placed on a perforated plate that is supported by a constriction in the desiccator wall. Lightly greased ground-glass surfaces provide a tight seal between the lid and the base of the desiccator. Whether it is being replaced or removed, the lid of the desiccator is properly moved by a sliding, rather than a lifting, motion. An airtight seal is achieved by slight rotation and direct downward pressure on the positioned lid.

When handling heated objects manipulations should be practiced first, if necessary, to assure that adequate control can be maintained with the implements to be used. If using tongs, always place them on surfaces so the grips are in the air rather than resting on the surface (prevents contamination). When a heated object is placed in a desiccator, the increased pressure of the enclosed air may be sufficient to break the seal between the lid and the base, causing the lid to slide off and break. Upon cooling, the opposite effect is

likely to occur, the interior of the desiccator now being under a partial vacuum. Both of these conditions can cause the contents of the desiccator to be physically lost or contaminated. Although it defeats the purpose somewhat, it may be best to allow some cooling to occur before finally sealing the lid. It also helps to break the seal several times during cooling to relieve any vacuum that may be forming.

6. Measurement of Liquids

The reliable measurement of volume is usually performed with the pipet, the buret and the volumetric flask (weighing is accurate but time-consuming). Pipets and burets are ordinarily designed and calibrated to deliver specified volumes, whereas volumetric flasks are calibrated on a "to contain" basis. Volumetric equipment is marked by the manufacturer to indicate not only the manner of calibration (usually with a TD for "to deliver" or a TC for "to contain") but also the temperature for which the calibration strictly refers. The volume occupied by a given mass of liquid varies with temperature, as does the volume of the container holding the liquid. As a general rule, volumetric glassware should not be heated because the calibration can be permanently altered.

6.1 Dispensing liquid reagents

Estimate the total volume of liquid reagent required, including any needed for rinsing of pipets or other volumetric glassware. Pour a slight excess of the reagent into a clean, dry beaker. (*Note: If the beaker is clean but not dry, pour into the beaker enough reagent to rinse the inside of the beaker by tilting the beaker and swirling the reagent. Dispose of this reagent. Repeat this rinsing at least two more times, for a total of at least 3 rinsings, and then pour a slight excess of the liquid reagent into the clean, rinsed beaker*). For pipetting, you will want to take sufficient excess to ensure that no air is taken up into the pipet, probably 15% - 30%.

6.2 Pipets

There are different types of pipets. Most of the work in this course involves the use of volumetric pipets which deliver a fixed volume of liquid and graduated pipets. Carefully inspect graduated pipets to see if the graduations extend to the tip of the pipet - if they do not then the volume of the tip is not calibrated and the pipet should be drained only as far as the lowest calibration line. Liquids are drawn into pipets through the application of a slight vacuum. **Never pipet anything by mouth**, use a pipet bulb.

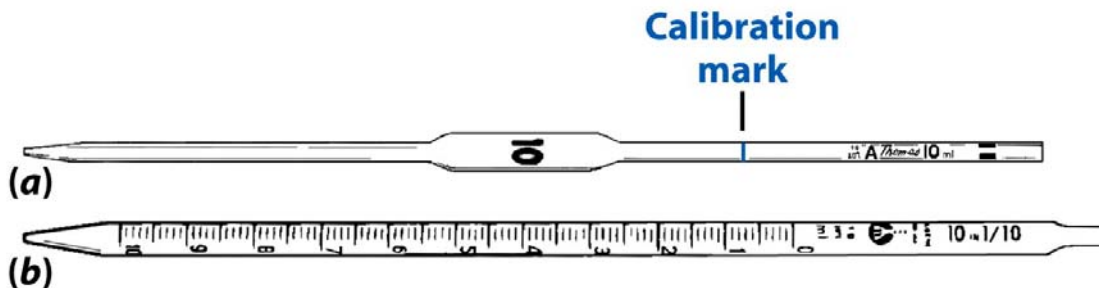


Figure 2-11
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Carefully inspect the pipet for damage, especially the tip. Consult your lab instructor if in doubt about damaged pipets.

In general, when planning dilutions, attempt to use the largest volumetric pipet that is practical. For example, making a 1/10 dilution with a 5 mL pipet and 50 mL flask will be less accurate than making the same dilution using a 10 mL pipet and a 100 mL flask. For even better accuracy, you could use a 25 mL pipet and a 250 mL flask! These guidelines apply primarily to your standard solutions, and particularly to any that will be used for subsequent dilutions. If in doubt about your 'dilution plan', speak to your instructor before the lab.

1. Use volumetric transfer pipets to carry out dilutions of standard solutions.
2. Rinse the pipet: Draw a small quantity of the liquid to be pipetted into the pipet. Tip the pipet nearly horizontal, and rotate to thoroughly wet the interior surface, up to above the mark. Discard the liquid and repeat rinsing at least twice more, for a total of at least three rinsings.
3. Carefully fill the pipet somewhat past the mark. Quickly place a forefinger over the upper end of the pipet to hold the liquid. Ensure that there are no bubbles in the bulk of the liquid or foam at the surface.
4. Tilt the pipet slightly from the vertical, and wipe the exterior free of adhering liquid.
5. Slowly allow the sample to drain into a waste beaker by partially releasing the forefinger.
6. Halt further flow when the bottom of the meniscus touches the top of the graduation mark.
7. Place the tip of the pipet well into the receiving vessel, and allow the liquid to drain. When free flow ceases, rest the tip of the pipet against the inner wall or bottom of the receiving vessel for 10 s.
8. Finally, withdraw the pipet with a rotating motion to remove any droplet still adhering to the tip. The small volume remaining **is not** blown out or rinsed into the receiving vessel.

Thoroughly rinse the pipet with distilled water after use.

Note that only the upper part of the pipet should be handled. Do not touch or hold the bottom of the pipet to avoid contaminating the liquid being pipetted (and yourself!).

Table 1: Tolerances of Class A transfer (volumetric) pipets

Volume (mL)	Tolerance (mL)	Tolerance as a % of total volume
0.5	± 0.006	1.2
1	± 0.006	0.6
2	± 0.006	0.3
3	± 0.01	0.33
4	± 0.01	0.25
5	± 0.01	0.2
10	± 0.02	0.2
15	± 0.03	0.2
20	± 0.03	0.15
25	± 0.03	0.12
50	± 0.05	0.1
100	± 0.08	0.08

6.3 Micropipettes

Micropipettes are commercially available in various sizes; some deliver a fixed volume while others are adjustable within a given range. Common volumes for micropipettes in an analytical lab are 5 μL to 2000 μL . Micropipettes use disposable plastic tips. To prevent contamination of these tips, insert the clean pipetter (not your hand) into bag containing the tips and using your hand on the outside of the bag, slide a tip into place.

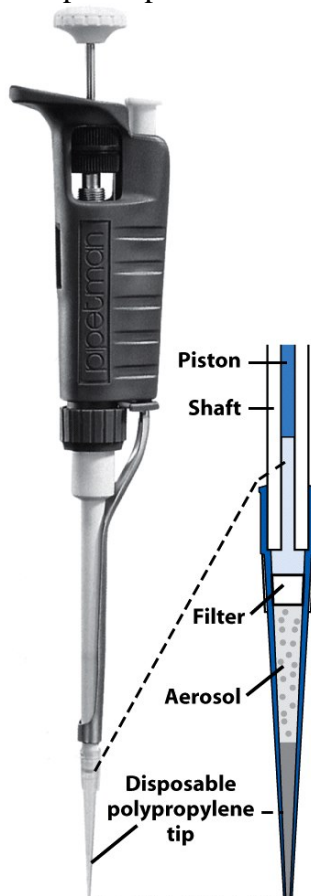


Figure 2-12ab
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Once the disposable tip is firmly in place, the micropipette is ready for use. Depress the button at the top of the micropipette to the first stop position and place the tip into the liquid to be transferred. Release the button to pull up the solution and then remove the micropipette. Place the tip into the receiving vessel and then depress the button at the top of the micropipette to the second stop position. Depressing to the first stop ejects most of the solution drawn up, while the second stop 'blows out' the remaining solution. Some micropipettes have a third stop position which ejects the tip.

Fixed volume micropipettes are more accurate than the adjustable micropipettes. Typical values for accuracy and precision are given for Eppendorf™ brand micropipettes.

Table 2: Fixed volume micropipette specifications

Pipette volume (μL)	Accuracy (%)	Precision (%)
5	± 1.5	< 0.8
10	± 1.0	< 0.5
50	± 0.7	< 0.3
> 100	± 0.6	< 0.2

6.4 Measurement of liquids using a graduated cylinder

If the reagent bottle is small and/or easy to handle, the amount of reagent required may be poured directly into the graduated cylinder. Immediately replace the cap or stopper on the reagent bottle. Clean up any spills as noted above and return the reagent bottle. Any excess reagent can be removed from the graduated cylinder using a pasteur pipet; this excess is then disposed of. If the reagent bottle is large, difficult to handle or if you are worried about spillage (especially if the reagent is a concentrated caustic, for example) the reagent should be poured into a clean beaker and then from the beaker into the graduated cylinder. In this instance, excess reagent from the graduated cylinder is returned to the beaker. Since graduated cylinders are not used for exacting analytical measurements, the small amount of water remaining after cleaning normally does not need to be removed by drying or rinsing.

6.5 Volumetric Flasks

Fill the flask until the bulb of the flask is almost full. Stop and swirl the solution to achieve adequate mixing. Bring the liquid level almost to the mark and allow time for solutions to drain from the neck of the flask (and for thermal expansion to room temperature, if necessary). Use a pasteur pipet to carefully dilute to the mark. Firmly stopper the flask, and invert repeatedly (do not shake) to assure uniform mixing. For storage beyond one day, transfer the contents to a clean, dry storage bottle or one that has been thoroughly rinsed with several portions of the solution from the flask. If you are using aqueous solvents, it is unnecessary to dry glassware; rinse glassware with deionized water. In general, volumetric glassware (pipets and volumetric flasks) should not be placed in a hot oven to dry as the thermal expansion may distort accuracy.

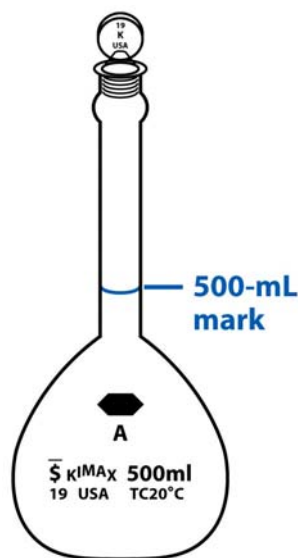


Figure 2-9a
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Table 3: Tolerances of Class A volumetric flasks

Volume (mL)	Tolerance (mL)	Tolerance as a % of total volume
1	± 0.02	2
2	± 0.02	1
5	± 0.02	0.4
10	± 0.02	0.20
25	± 0.03	0.12
50	± 0.05	0.1
100	± 0.08	0.08
200	± 0.1	0.05
250	± 0.12	0.048
500	± 0.2	0.04
1000	± 0.3	0.03
2000	± 0.5	0.025

6.6 Burets

Burets are designed to deliver liquids primarily for the purpose of volumetric titrations. They are still widely used in an analytical laboratory and considered a 'reference' method for many analytes. A standard 50 mL buret is divided into 1 mL graduations with 0.1 mL sub-divisions. Leveling the eye with the bottom of the meniscus, an analyst should be able to interpolate volume readings to within 0.02 mL with a high degree of reproducibility.

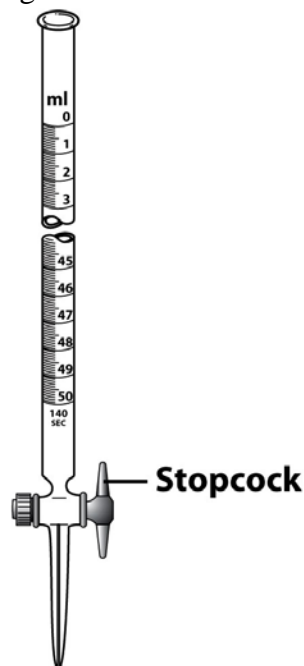


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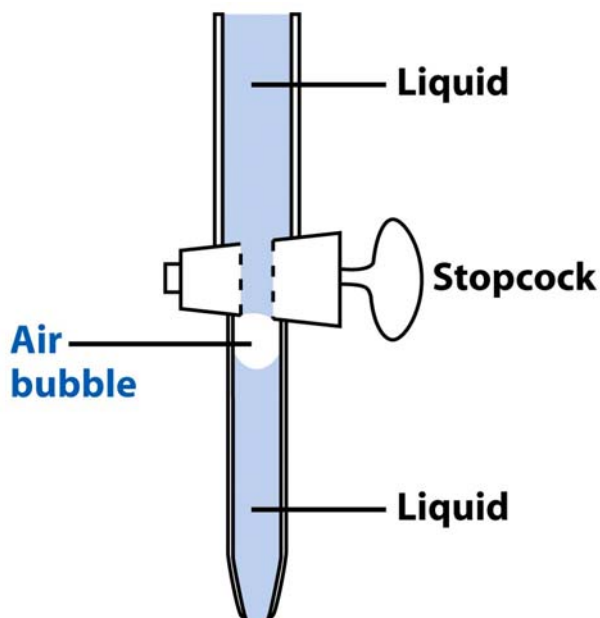


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Filling burets

- Load 50 mL burets using a funnel .
- Rinse inside of buret including the valve and tip, with three small portions of titrant to be used.
- Fill the buret and run some titrant into a waste beaker checking for air bubbles in the tip.
- Make sure all air bubbles have cleared.
- Let the solution level stabilize and record the initial volume to nearest ± 0.02 mL.

Titrating

- Control the stopcock valve with your non-dominant hand. This allows for finer control of the valve and swirling of the receiving flask with your dominant hand.
- If you know (by calculation or experience) the approximate volume to be delivered, you can add ~80% quickly and then slow down as you approach the end point.
- If the end-point is to be determined by a colour change, you will observe a temporary change which disappears with swirling as you get close to the endpoint.
- To add less than a drop of titrant as the endpoint approaches, carefully adjust the stopcock valve until titrant just begins to flow. Close the valve and wash the hanging drop into the receiving flask with a washbottle.

- Repeat the above step until the endpoint colour change is just barely visible and permanent for more than 30 sec.

6.7 Digital titrators

Digital titrators are small portable devices designed to deliver minute volumes of titrant. They are convenient for field measurements (although less precise).

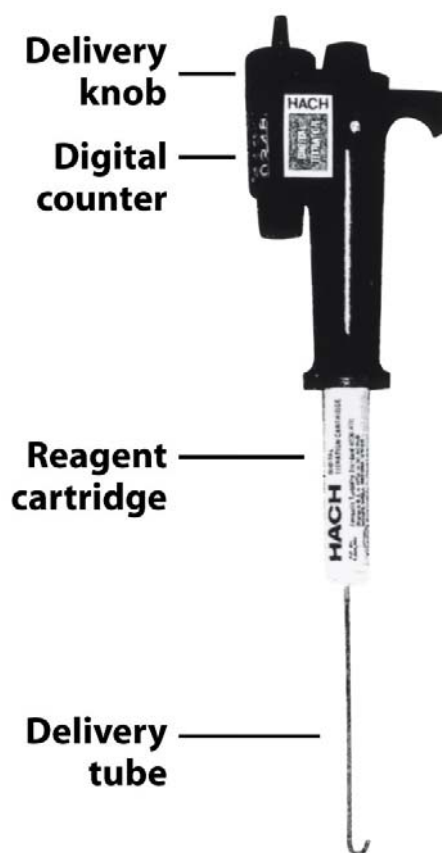


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Follow the instructions below for their use:

1. Choose the appropriate titrant Reagent Cartridge for the analyte and method chosen. Be sure to check both the titrant identity and concentration. Record the sample volume and the digit multiplier to be used.
2. With the titrator plunger fully retracted, slide the Reagent Cartridge into place.
3. Remove the Reagent Cartridge tip cap and replace with a Delivery Tube.
4. Depress the button on the plunger slider and slide the plunger until it meets resistance.

5. With the titrator in the vertical position (tip up) continue to slide the plunger by hand or by cranking the Delivery Knob to remove all air bubbles from the Reagent Cartridge and the Delivery tube. This will require wasting some of the titrant.
6. Wipe the outside of the Delivery Tube with a Kimwipe™ to remove excess titrant.
7. Re-set the Digital Counter to zero.
8. Titrate the sample to the specified end-point.
9. Record the number on the Digit Counter and convert this to concentration of analyte using the multiplier appropriate for the particular method.

7. Quality Control Program

For most experiments, a 'quality control' sample, or QC, will be available and labeled as either a Certified Reference Material (CRM) or a Standard Reference Material (SRM). You will determine the concentration of analyte in the QC and compare that value to the known value. Accuracy is expressed as a %bias or % error and give insight into the experimental method and/or the analyst.

Replicates are required or recommended in most cases to assess precision and are typically reported as relative standard deviations (RSD) or 95% confidence limits (CL's)

8. Sample Sequencing

Analytical laboratories have strict guidelines for sample analysis, which are required to meet various quality control standards. For CHEM 311, you will use the following sequence in all experiments, unless otherwise noted. In some cases, you may not have separate calibration and method blanks. Try to run at least one sample in triplicate; this will allow you to calculate precision estimates.

Table 4. GLP Order for Sample and Standard Analysis

Run #	Sample	Reason
1	calibration blank	set the instrument zero, or check for impurities
2	most conc. standard	make sure the instrument range is set properly
3	least conc. standard	rinse well before running this one!
4	remaining standards	
5	calibration blank	make sure instrument zero hasn't drifted
6	method blank	check sample preparation for contamination
7	samples ('unknown')	run the samples!!
8	QC sample	use to confirm the instrument is working properly, and standards are made correctly.
9	most conc. standard	make sure instrument response hasn't drifted

Good Laboratory Practices Exercise**Objective:**

- a) to prepare a stock and standard solution by volumetric dilution and
- b) to standardize a solution of unknown concentration using the standard solution.

Procedure:

An accurately weighed quantity of sulfamic acid (a primary standard) is provided in a 100 mL volumetric flask. Prepare your stock solution of sulfamic acid (HSO_3NH_2) by diluting this solid to the 100.00 mL mark with deionized water. Ensure complete mixing of this solution with a minimum of 15-20 inversions. Using the mass information provided on the flask, determine the molarity of stock sulfamic acid.

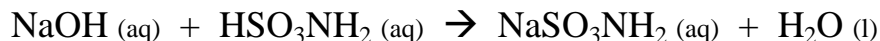
($\text{HSO}_3\text{NH}_2 = 97.10 \text{ g/mol}$)

$$[\text{HSO}_3\text{NH}_2]_{\text{Stock}} =$$

Next, prepare a ~0.1M standard solution of sulfamic acid by diluting the appropriate volume of stock solution with deionized water in a 100 mL volumetric flask. Note: the final solution should be known as precisely as possible (it is a standard solution), but need not be exactly 0.1000M (i.e., 0.09981 M or 0.1022 M are completely acceptable values).

$$[\text{HSO}_3\text{NH}_2]_{\text{Standard}} =$$

Finally, *standardize* the unknown sodium hydroxide solution provided by titrating a 25 mL aliquot of standard sulfamic acid with the NaOH solution. Use 3 drops of indicator solution (phenolphthalein) to visualize the endpoint of the titration by the appearance of a light persistent pink color. The reaction on question is:



Calculate the concentration of NaOH in the unknown, and if time permits, repeat the titration a few more times. How do your values for [NaOH] compare with the other groups?

[NaOH] =

Summary of Student Results from previous year for reference.
STANDARDIZATION OF NaOH (INTRO LAB EXERCISE)

Concentrations of NaOH in moles/L*

student group	[NaOH] (mol/L)			group mean (M)	group std dev (M)	group 95% CI (M)
	trial 1	trial 2	trial 3			
1	0.1100	0.1288	0.1194	0.1194	0.0094	0.0233
2	0.1320	0.1314	0.1322	0.1319	0.0004	0.0010
3	0.1295	0.1329	0.1328	0.1317	0.0019	0.0048
4	0.1318	0.1326	0.1333	0.1326	0.0008	0.0019
5	0.1327	0.1337	0.1346	0.1337	0.0010	0.0024
6	0.1283	0.1326	0.1332	0.1314	0.0027	0.0066
7	0.1297	0.1323	0.1310	0.1310	0.0013	0.0032

overall mean (M)	0.1302
std dev (M)	0.0049
95% CI (M)	0.0045

Final Reported [NaOH] = 0.130₂ +/- 0.005 moles/L

* from the titration of 25.00 mL of ~0.1 M sulfamic acid standard solutions using a phenolphthalein end-point