A Study on the Types of Assay Used in the British Pharmacopoeia

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis/project titled "A Study on the Types of Assay Used in the British Pharmacopoeia" submitted by Farhan Sadit (17346019) of Spring, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on 1st September, 2021.

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Ethics Statement

This study does not involve any kind of human or animal trial.

Abstract

In this study, the most common assay type for the APIs in British Pharmacopoeia monographs was determined. In addition, any possible patterns in solubility, Log P and pKa was investigated. Acid base titration, non-aqueous titration and HPLC were found to be the most common assay types. Also found some other analytical methods like complexometric titration, redox titration, UV-Vis spectroscopy, microbiological assay have been recommended. Some pattern in case of solubility was found only; log P and pKa do not follow any specific orientation based on assay type.

Keywords: Assay; Analysis; Titration; Method; Pharmacopoeia; Pattern

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List of Acronyms

HPLC	High Performance Liquid Chromatography
UV	Ultraviolet

Glossary

Assay	An assay is a procedure for determining the composition or quality of a substance.

Chapter 1

Introduction

1.1 Literature Review

BP or British pharmacopoeia, which is the United Kingdom's national pharmacopoeia, is a collection of authoritative and widely accessible quality standards for medicinal substances, followed up by guidance and additional value-adding information. It was first published in the year 1864. Since then it has become a vital part of the United Kingdom’s Medicines and Healthcare products Regulatory Agency (MHRA) and continuously evolving to adapt with the changing world to include new medicines, technology and emerging science. (Pound, 2017)

The most fundamental analytical tool for a compound is the mass of that particular compound and gravimetry is the oldest analytical technique. It includes all the analytical methods and the basic concept of this technique is measuring the mass or change in the mass of the sample. Gravimetric analysis works on the idea of determining the mass of an ion in a pure compound and then using that information to calculate the mass percent of that same ion in a known quantity of an impure compound. There are four types of gravimetric methods. Gravimetry is still used in specialized applications, despite the fact that it is no longer the most important analytical approach. (Lowell & Shields, 1991)

Acid base titration is one of the most common types of qualitative analysis. It is an analytical method depending on neutralization of an acid and base when they are mixed in a solution. An indicator is used which helps the process of identification or quantification of the sample by changing the color of the solution at the endpoint of the titration. It is a standard pharmacopoeial method for the assay of unformulated drugs and excipients and some

formulated drugs and also used for standardizations of raw materials and intermediates used in drug synthesis. This method is widely used because of its accuracy, robustness and capability of higher degree of precision than other instrumental analysis methods. Apart from this, one of the major advantages of this method is, this method can be automated. Moreover, it is an absolute method and instrument calibration is not necessary for the procedure. Also, as it does not require any specialized instrument, it is a less expensive method to perform for analytical purposes. Though having these as advantages it has also some drawbacks. One of the major problems of this method is, if the method is not automated, it becomes time consuming and technical skill of the operator becomes a great factor on accuracy of the method. Moreover, this method requires a comparatively larger quantity of sample and reagents than other analytical methods. Also, it is a non-selective method. (Pierre, 2019; Watson, 2006)

Non aqueous titration is a special type of titration method where analyte substances is dissolved in a solvent which does not contain any water. It is the most common titrimetric procedure in pharmacopoeial assay. Non aqueous titration is the most common analytical method for organic compounds and weak acids and bases because of its water free state and capability of providing a suitable solvent for the organic compounds. Water has the properties of both a weak acid and a weak base, therefore it can efficiently compete with very weak acids and bases for proton donation and acceptance in an aqueous solution. To get rid of this problem, organic solvent is used in non-aqueous titration to replace water as solvent as they are less competent with weak acids and bases unlike water in case of proton donation and acceptance. Major advantage of this method is, samples which are insoluble in water can be dissolved in organic solvent and assayed by this method. It is a simple method to operate with greater rate of accuracy. However, it also has some disadvantages like aqueous method. Like aqueous titration it also requires a large quantity of sample and reagent. Additionally, the

environment also needs to be controlled. For example, temperature and moisture need to be in controlled range. Also, volatile substances are used in non-aqueous titration which is responsible for polluting the environment. (Watson, 2006)

Complexometric titration is a type of volumetric analysis in which the endpoint is determined by forming a colored complex. Complexometric titrations are very effective for determining the concentration of a combination of metal ions in solution. EDTA (Ethylenediaminetetracetic Acid) is used as titrant which forms a stable complex with almost every metal except a few like Sodium and potassium. The end-point of the titration is commonly detected using an indicator that produces an evident color shift. It is basically useful for detecting metal ions because of its simple procedure and cost effective technique. Endpoint can be determined visually and does not require any expensive materials or apparatus. However, for being a destructive method large amounts of samples and reagents are wasted. Also, sometimes temperature, pH and humidity can affect the results for being an open system. (Watson, 2006)

Redox titration is a type of titration where an oxidizing agent (or oxidant) is titrated with a reducing agent (or reductant) or vice versa. For the reaction to complete with a sharp end point, there must be a sufficiently large difference between the oxidizing and reducing capabilities of these agents. The oxidation process results in the loss of electrons, whereas the reduction process results in the gain of electrons. As a result, an oxidizing agent is one that receives electrons, whereas a reducing agent is one that loses electrons. By removing electrons from the other substance, an oxidizing agent oxidizes it. By contributing electrons to the other substance, a reducing agent reduces it. Oxidation and reduction reactions always happen at the same time. It is impossible to take place in the absence of the other. The oxidizing agent gets reduced while the reducing agent experiences oxidation during a redox reaction. (Marie, 2015)

Precipitation titration is one of the oldest titration methods. Basic idea of this method is to analyze the sample by forming a precipitation. In precipitation titration, the titrant reacts with analyte and forms an insoluble substance called precipitate. It continues till the last amount of analyte is consumed. A rapid change in a physical state of the solution marks the titration's end point. (Abbaspour & Khajehzadeh, n.d.)

UV-Vis spectroscopy is an analytical method that analyzes the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. In this method, radiation of 200-700nm wavelength is passed through the compound solution. The electrons in the bonds within the molecule are stimulated to a higher quantum state, absorbing a significant amount of energy traveling through the solution in the process. The amount of the absorbed energy depends on the bond strength. If the electrons are held loosely in the bond of the molecule, it will absorb the longer wavelength radiation. UV-Vis spectroscopy is used for various purposes like quantification of drugs in different formulations, determining pKa values of some drug, checking physico-chemical properties of medicine etc. Also it helps to determine drug release profile in dissolution testing, to understand reaction kinetics of drug degradation. Pharmacopoeial identity check is also an application of UV-Vis spectroscopy. It is widely used because of its user-friendly procedure. After having such advantages, it also has some drawbacks. For example, this method cannot be used to analyze the mixture solution and it is moderately selective. (Watson, 2006)

HPLC(High-Performance Liquid Chromatography) is the most common technique which is used for quantification of drugs in different formularies. It is a separation based method involving a liquid mobile phase and solid stationary phase. Liquid mobile phase is passed through the stationary phase in a stainless steel column with an approximate diameter of 3-10µm. In combination with UV-Vis detection, HPLC provides a unique, precise and accurate

quantitative analytical result for the pharmaceutical products which is the standard method for the worldwide pharmaceutical industries. Also, stability of drug products can be monitored by this method. Moreover, HPLC can be used in measuring drugs and their byproducts in biological fluid and protein binding of drugs. HPLC method is widely used because of its automated, well controlled and precise technique which helps to get a highly standard value. Again, no involvement of heat makes it a sample degradation free method unlike gas chromatography. There are a variety of columns and detectors which helps to adjust the selectivity of the method. Apart from these advantages, this technique is highly expensive because of the apparatus like detectors and disposal of the large amount of organic waste that is produced during assay. Moreover, drugs that need to be analyzed have to be extracted from the formulation before the analysis which makes it a little bit of a complex method. (Watson, 2006)

Microbiological assay or bioassay are the process to analyze the impact of any API or compound on micro-organisms. There are several analytical techniques to quantify the concentration of the antibiotic in the body fluid which might be considered as microbiological assay. It helps to select an effective antibiotic for patient recovery. However nowadays, different automated assays instead of classical microbiological assays are being popular among the generic manufacturers due to their accuracy and speed. It is because microbiological assay is unable to quantify other substances except API in the same matrix. (Zuluaga et al., 2009).

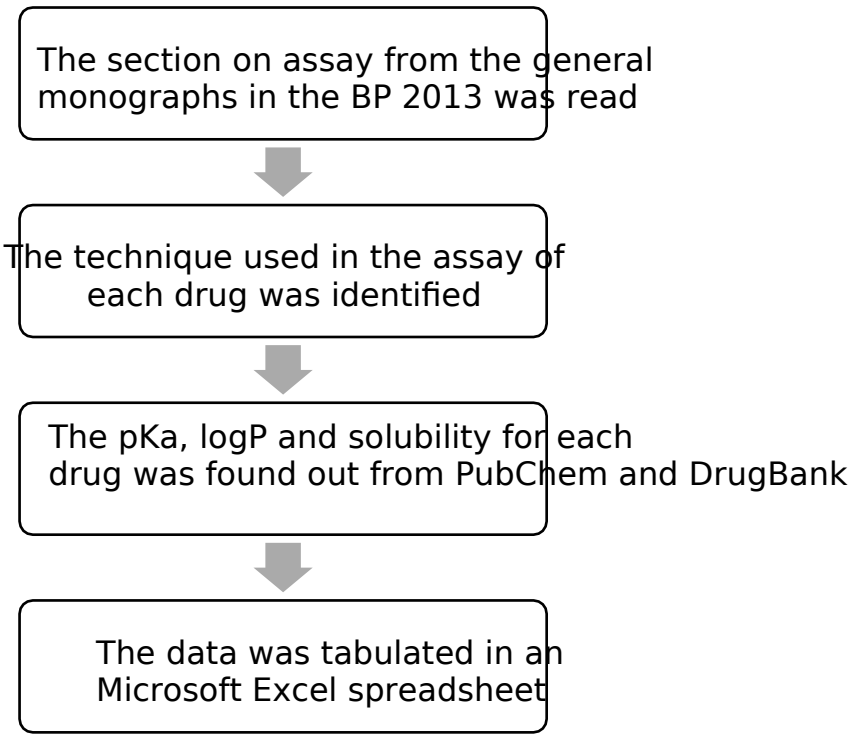
1.2 Aims

The objective of this analysis was to find out the most common assay techniques used in the monographs in British Pharmacopoeia and also whether there is any pattern present or not in the log P, pKa and solubility of drugs being assayed by the same technique.

Chapter 2

Method

The following method was performed for this thesis.



Chapter 3

Results

3.1 Overview

A total of 220 monographs were read from the BP 2013. Monographs were read in an alphabetical order.

Seven different analytical techniques have been recommended for these 220 drugs. The most common analytical techniques recommended were acid base titration, non-aqueous titration and HPLC. 51 API were found to be assayed by acid base titration; 72 by non-aqueous titration; and 71 by HPLC. Rest of the drugs were assayed by complexometric titration (5), precipitation titration (3), redox titration (6), UV-Vis Spectroscopy (7) and others (5).

3.2 Aqueous acid base titration

The following table lists the drugs assayed by aqueous acid base titration.

Table 1List of drugs assayed by aqueous acid base titration

Sl.	Drug Name	pKa	Log P	Solubility	Subtype	Titrant
1	Acamprosate Calcium	Strong acidic: -1.2, Strong basic: 4.4	-2.3	Freely soluble in water, 1.97mg/ml	Direct titration	Sodium Hydroxide
2	Acebutolol Hydrochloride	9.46	1.71	In ethanol 70mg/ml, In water, 200mg/L	Direct titration	Sodium Hydroxide
3	Aceclofenac	4.157	2.17	insoluble in water, 96% soluble in ethanol	Direct titration	Sodium Hydroxide

4	Acemetacin	2.6	4.49	insoluble in water, slightly soluble in ethanol	Direct titration	Sodium Hydroxide
5	Acenocoumarol	4.7	1.98	In water, 9.39mg/L,	Direct titration	Sodium Hydroxide

				soluble in alcohol		
6	Acetylcholine chloride	Strong base: -7	-4.2	>27.2(µg/mL)	Back titration	Hydrochlori c Acid
7	Alfentanil Hydrochloride	6.5	2.16	34.6 mg/L	Direct titration	Sodium Hydroxide
8	Alprenolol Hydrochloride	Strong Acidic: 14.09, Strong basic: 9.67	3.1	547 mg/L	Direct titration	Sodium Hydroxide
9	Amantadine Hydrochloride	10.71	2.53	0.0846 mg/mL	Direct titration	Sodium Hydroxide
10	Ambroxol Hydrochloride	Strong Acidic: 15.26, Strong basic: 9.01	3.72	0.0185 mg/mL	Direct titration	Sodium Hydroxide
11	Amiloride hydrochloride	Strong Acidic: 11.43, Strong basic: 7.35	-0.48	less than 1mg/mL at 67.1°F	Direct titration	Sodium Hydroxide
12	Aminobenzoic Acid	2.38	0.83	6110mg/L at 30°C	Direct titration	Sodium Hydroxide
13	Aminophylline	Strong Acidic: 7.82, Strong Basic: -0.78	-3.03	200000mg/L	Direct titration	Hydrochlori c Acid
14	Amiodarone Hydrochloride	6.56	7.2	In watee, 700mg/l at 25°C	Direct titration	Sodium Hydroxide
15	Amitriptyline Hydrochloride	9.4	4.92	>47.1(µg/mL)	Direct titration	Sodium Hydroxide
16	Apomorphine Hydrochloride Hemihydrate	Strong Acidic: 6.58, Strong Basic: 13.25	2	0.51 mg/mL	Direct titration	Sodium Hydroxide
17	Articaine Hydrochloride	Strong Acidic: 11.61, Strong Basic: 8.68	1.93	0.0285 mg/mL	Direct titration	Sodium Hydroxide
18	Aspirin	3.5	1.18	10 mg/mL	Direct titratio	Hydrochlori c Acid

					n	
19	Bambutero l Hydrochlori de	Strong Acidic: 13.91, Strong Basic: 9.52	1.69	0.469 mg/mL	Direct titratio n	Sodium Hydroxi de
20	Benzbromaron e	Strong Acidic: 5.11 Strong Basic: -3.8	5.52	0.0131 mg/mL	Direct titratio n	Sodium Hydroxi de
21	Benzyl benzoate	-6.9	3.97	15.4 mg/L	Direct titratio n	Hydrochl ori c Acid
22	Betahistine Dihydrochlorid e	3.5	0.1	49.3 mg/mL	Direct titratio n	Sodium Hydroxide
23	Betaxolol Hydrochloride	9.4	2.81	451 mg/L	Direct titratio n	Sodium Hydroxide
24	Bezafibrate	Strong Acidic: 3.83, Strong Basic: -0.84	3.97	1.55e-03 g/L	Direct titratio n	Sodium Hydroxi de
25	Bromhexin e Hydrochlori de	8.69	5.14	<1 mg/mL	Direct titratio n	Sodium Hydroxi de

26	Busulfan		-0.52	69000 mg/L	Direct titration	Sodium Hydroxide
27	Carbasalate Calcium	Strong acidic: 3.41, Strong basic: -7.1	3.17	Freely soluble in Water	Direct titration	Hydrochlori c Acid
28	Carisoprodol	15.06	2.1	less than 1 mg/mL at 67.1°F	Direct titration	Hydrochlori c Acid
29	Carteolol Hydrochloride	Strong Acidic: 13.41, Strong basic: 9.76	1.1	0.421 mg/mL	Back titration	Sodium Hydroxide
30	Celiprolol Hydrochloride	Strong Acidic: 13.55, Strong basic: 9.66	2.29	0.174 mg/mL	Back titration	Sodium Hydroxide
31	Cetrizine Hydrochloride	1.52, 2.92 and 8.27	2.8	101 mgL	Direct titration	Sodium Hydroxide
32	Chenodeoxycholi c Acid	Strong Acidic: 4.6, Strong basic: -0.54	4.15	89.9 mg/L	Direct titration	Sodium Hydroxide
33	Chloral Hydrate	Strong Acidic: 9.51, Strong basic: -5.1	0.99	793000 mg/L	Direct titration	Sulfuric Acid
34	Chlorambucil	5.75	1.7	12400 mg/L	Direct titration	Sodium Hydroxide
35	Chlorocyclizine Hydrochloride	7.63	4.16	0.0424 mg/mL	Direct titration	Sodium Hydroxide
36	Chlorpromazine Hydrochloride	9.3	5.41	greater than or equal to 100 mg/mL at 75° F	Back titration	Sodium Hydroxide
37	Chlorpropamide	5.13	2.27	less than 1 mg/mL at 57° F	Direct titration	Sodium Hydroxide
38	Chlorprothixene Hydrochloride	9.76	5.18	0.295 mg/mL	Back titration	Sodium Hydroxide
	Choline	Strong Acidic:			Back	Sodium

39	theophyllinate	7.82, Strong Basic: -0.78	-0.99	3.84 mg/mL	titration	Hydroxide
40	Ciclopirox	Strong Acidic: 6.84, Strong basic:-6.2	2.3	1.41e+00 g/L	Direct titration	Sodium Hydroxide
41	Cilastatin Sodium	Strong Acidic; 2.53, Strong basic:9.14	-0.29	0.1 mg/mL	Back titration	Sodium Hydroxide
42	Cilazapril	Strong Acidic: 3.41, Strong basic: 5.35	0.8	0.5 g/100 mL	Direct titration	Sodium Hydroxide
43	Cimetidine Hydrochloride	6.8	0.4	9380 mg/L (at 25 °C)	Direct titration	Sodium Hydroxide
44	Cincocaine Hydrochloride	8.85	4.4	>57 [ug/mL]	Direct titration	Sodium Hydroxide
45	Ciprofibrate	Strong Acidic: 3.69, Strong Basic: -4.9	3.97	0.00779 mg/mL	Direct titration	Sodium Hydroxide

46	Citalopram Hydrobromide	9.78	3.76	0.00588 mg/mL	Back titration	Sodium Hydroxide
47	Clenbuterol Hydrochloride	Strong Acidic: 14.06, Strong Basic: 9.63	2.94	46.5 [ug/mL]	Direct titration	Sodium Hydroxide
48	Clomipramine Hydrochloride	9.2	5.19	0.294 mg/L	Direct titration	Sodium Hydroxide
49	Clopidogrel Hydrogen Sulphate	5.3	3.8	51 mg/L at 25 °C	Direct titration	Sodium Hydroxide
50	Cocaine Hydrochloride	8.61	2.3	1800 mg/L at 22° C	Direct titration	Sodium Hydroxide
51	Codeine Hydrochloride	8.2	1.39	0.577 mg/mL	Direct titration	Sodium Hydroxide

3.3 Non aqueous titration

The following table lists the drugs assayed by non aqueous titration.

Table 2 List of drugs assayed by non aqueous titration

Sl.	Drug Name	pKa	LogP	Solubility	Subtype	Titrant
1	Acetazolamide	7.2	-0.26	980ml/L at 30° C	Direct titration	Ethanolic Sodium Hydroxide
2	Aciclovir	2.52 and 9.35	-1.76	1.41mg/mL at 25°C	Direct titration	Perchloric Acid
3	Adenosine	pKa1=3.6 ; pKa2=12.4	-1.05	8230 mg/L	Direct titration	Perchloric Acid
4	Adrenaline/ Epinephrine	8.59	-1.37	In water, 180mg/L at 20°C	Direct titration	Perchloric Acid
5	Albendazol	6.9	2.7	In water, 4.1×10	Direct titratio	Perchloric Acid

				+1 mg/L at 25°C	n	
6	Alcuroniu m Chloride	Strong acidic: 15.3, strong basic: 1.52	-4.3	Freely soluble in water	Direct titratio n	Perchloric Acid
7	Alfuzosin Hydrochloride	8.13	1.4	in water, 92 mg/L	Direct titratio n	Perchloric Acid
8	Alimemazi ne Tartrate	9.05	4.71	0.942 mg/L	Direct titratio n	Perchloric Acid
9	Alprazolam	Strong acidic: 18.3, strong basic: 5.08	2.12	In water, 13.1 mg/L at 25° C	Direct titratio n	Perchloric Acid
10	Alverine Citrate	10.44	5.73	0.00096 mg/mL	Direct titratio n	Perchloric Acid
11	Amfetamine Sulphate	10.13	1.76	In water, 2.8×10+4	Direct titratio n	Perchloric Acid

				mg/L at 25°C		
12	Aminoglutethimid e	Strong Acidic: 11.69, Strong basic: 4.28	1.3	In water, 2.49×10+3mg /L at 25°C	Direct titration	Perchloric Acid
13	Amisulpride	9.37	1.06	2.93e-01 g/L	Direct titration	Perchloric Acid
14	Amobarbital	7.84	2.07	less than 1 mg/mL at 65.3°F	Direct titration	Ethanolic Sodium Hydroxide
15	Amobarbital Sodium	7.84	2.07	603 mg/L at 25°C	Direct titration	Ethanolic Sodium Hydroxide
16	Antazoline Hydrochloride	4.9	3.38	>45.3(µg/mL)	Direct titration	Alcoholic Potassium Hydroxide
17	Atenolol	9.6	0.16	13300 mg/L	Direct titration	Perchloric Acid
18	Atropine Sulphate	9.43	1.83	2200 mg/L at 25°C	Direct titration	Perchloric Acid
19	Azapropazone	Strong Acidic: 0.52, Strong Basic: 7.58	0.92	0.641 mg/mL	Direct titration	Perchloric Acid
20	Azathioprine	7.87	0.1	less than 1 mg/mL at 73°F	Direct titration	Tetrabutylammoni um Hydroxide
21	Azelastine Hydrochloride	8.88	4.04	In Water, 5.12×10-2 mg/mL at 25°C	Direct titration	Perchloric Acid
22	Baclofen	9.62+0.1(amin o group) & 3.87+0.1(carbo xyl group)	1.3	less than 1 mg/mL at 64°F	Direct titration	Perchloric Acid
23	Barbital	8.14	0.65	7460 mg/L	Direct titration	Ethanolic Sodium Hydroxide

24	Bendroflumethaiz ide	8.5	1.89	108 mg/L at 25°C	Direct titration	Tetrabutylammoni um Hydroxide
25	Benperidol	Strong Acidic: 11.67, Strong Basic: 8.55	3.91	0.0306 mg/mL	Direct titration	Perchloric Acid
26	Benserazide Hydrochloride	Strong Acidic: 8.66, Strong Basic 7.48	-2.3	35.4(µg/mL)	Direct titration	Perchloric Acid
27	Benzydamine Hydrochloride	9.26	3.78	22.5(µg/mL)	Direct titration	Perchloric Acid
28	Bifonazole	6.69	4.77	2.45e-03 g/L	Direct titration	Perchloric Acid
29	Biotin	Strong Acidic: 4.4, Strong Basic: -1.9	0.5	220 mg/L	Direct titration	Tetrabutylammoni um Hydroxide
30	Biperidin Hydrochloride	Strong Acidic: 13.82, Strong	4.25	25.1 mg/L	Direct titration	Alcoholic Potassium

		Basic: 9.3				Hydroxide
31	Bisoprolol Fumarate	9.5	2.2	0.0707 mg/mL	Direct titration	Perchloric Acid
32	Bretylium Tosilate	17.58	-1.4	>62.2(µg/mL)	Direct titration	Perchloric Acid
33	Bromazepam	Strong Acidic: 12.24, Strong Basic: 2.68	2.05	3.99e-02 g/L	Direct titration	Perchloric Acid
34	Bromocriptine Mesilate	Strong Acidic: 9.69, Strong Basic: 6.71	3.2	0.0858 mg/mL	Direct titration	Perchloric Acid
35	Bromperidol	Strong Acidic: 13.97, Strong Basic: 8.07	3.78	0.00723 mg/mL	Direct titration	Perchloric Acid
36	Brompheniramine Maleate	9.48	3.4	0.0127 mg/mL	Direct titration	Perchloric Acid
37	Brotizolam	Strong Acidic: 18.49, Strong Basic: 3.9	2.79	0.058 mg/mL	Direct titration	Perchloric Acid
38	Buclizine Hydrochloride	8.04	7.1	0.000246 mg/mL	Direct titration	Perchloric Acid
39	Bufexamac	Strong Acidic: 8.86, Strong Basic: -4.8	2.08	>33.5(µg/mL)	Direct titration	Lithium Methoxide
40	Bumetanide	Strong Acidic: 4.69, Strong Basic: 2.7	2.6	>20mg/mL	Direct titration	Sodium Hydroxide
41	Bupivacaine Hydrochloride	8.1	3.41	2400 mg/L at 25°C	Direct titration	Sodium Hydroxide
42	Buprenorphine	8.31	4.98	1.68e-02 g/L	Direct titration	Perchloric Acid
43	Buspirone Hydrochloride	7.62	2.63	0.588 mg/mL	Direct titration	Perchloric Acid

44	Caffeine	14	-0.07	2.17 g/100mL	Direct titration	Perchloric Acid
45	Calcium Pantothenate	Strong Acidic: 4.35, Strong Basic: -2.8	-1.1	60.5 mg/mL	Direct titration	Perchloric Acid
46	Candesartan Cilexetil	Strong Acidic: 4.23, Strong Basic: 1.45	6.1	Insoluble in water	Direct titration	Perchloric Acid
47	Carbachol	15.23	-3	In water, 1g/mL	Direct titration	Perchloric Acid
48	Carbenoxolone Sodium	Strong Acidic: 4.04, Strong basic: -5.1	5.46	Freely soluble in water	Direct titration	Tetrabutylammoni um Hydroxide
49	Carbidopa	2.3	-1.9	3.8 mg/L	Direct titration	Perchloric Acid
50	Carbocisteine	1.84	-4.24	1.6g/L	Direct titration	Perchloric Acid

51	Carvedilol	Strong Acidic: 14.03, Strong basic: 8.74	3.8	88mg/mL	Direct titration	Perchloric Acid
52	Chlorhexidine Acetate	10.8	0.08	800 mg/L at 20° C	Direct titration	Perchloric Acid
53	Chlorhexidine Gluconate solution	pKa1 = 7.63; pKa2 = 9.92; pKa3 = 8.22; pKa4 = 10.52	0.08	In water, 800 mg/L at 20 °C	Direct titration	Perchloric Acid
54	Chloroquine Phosphate	10.1	4.63	In water, 0.14 mg/L at 25 °C	Direct titration	Perchloric Acid
55	Chloroquine Sulphate	10.1	4.63	0.0175 mg/mL	Direct titration	Perchloric Acid
56	Chlorphenamine Maleate	9.13	3.38	10 to 50 mg/mL at 70° F	Direct titration	Perchloric Acid
57	Chlorpromazine	9.3	5.41	2.55 mg/L	Direct titration	Perchloric Acid
58	Ciclopirox Olamine	Strong Acidic: 6.84, Strong basic:-6.2	2.3	1.41 mg/mL	Direct titration	Perchloric Acid
59	Cimetidine	6.8	0.4	5 mg/mL at 68° F	Direct titration	Perchloric Acid
60	Cinnarizine	8.1	5.77	0.00M	Direct titration	Perchloric Acid
61	Ciprofloxacin	6.09	0.28	<1mg/mL	Direct titration	Perchloric Acid
62	Clebopride Malate	Strong Acidic: 14.61, Strong basic: 8.52	2.69	0.0198 mg/mL	Direct titration	Perchloric Acid
63	Clemastine Fumarate	9.55	5.2	Very slightly soluble in water	Direct titration	Perchloric Acid

64	Clioquinol	Strong Acidic: 7.34, Strong basic: 3.28	3.66	less than 1 mg/mL at 68° F	Direct titration	Perchloric Acid
65	Clofazimine	8.51	7.66	0.225 mg/L	Direct titration	Perchloric Acid
66	Clomifene Citrate	9.31	7.2	0.000414 mg/mL	Direct titration	Perchloric Acid
67	Clonazepam	pK1 = 1.5; pK2 = 10.5	2.41	100 mg/L	Direct titration	Perchloric Acid
68	clonidine Hydrochloride	8.05	1.59	0.48 mg/mL	Direct titration	Sodium Hydroxide
69	Clopamide	Strong Acidic: 8.85, Strong basic: 1.32	2.33	0.139 mg/mL	Direct titration	Perchloric Acid
70	Clotrimazole	4.1	0.5	0.49 mg/L	Direct titration	Perchloric Acid
71	Clozapine	7.5	3.23	11.8 mg/L	Direct	Perchloric Acid

					titration	
72	Colchicine	1.85	1.3	greater than or equal to 100 mg/mL at 70° F	Direct titration	Perchloric Acid

3.4 Complexometric titration

The following table lists the drugs assayed by complexometric titration.

Table 3 List of drugs assayed by complexometric titration

Sl.	Drug Name	pKa	Log P	Solubility	Subtype	Titrant
1	Calcium Carbonate	6.05	0.47	insoluble in water	Direct titration	sodium edetate
2	Calcium Glucoheptone	Strong Acidic: 3.38, Strong Basic: -3	-1.9	39.8 mg/mL	Direct titration	sodium edetate
3	Calcium Gluconate	Strong Acidic: 3.39, Strong Basic: -3	-7.51	In Water, 3.3g/100cc at 15°C	Direct titration	sodium edetate
4	Calcium Lactate	Strong Acidic: 3.78, Strong Basic: -3.7	-0.36	48g/L	Direct titration	sodium edetate
5	Calcium Levulinate Dihydrate	Strong Acidic: 4.32, Strong Basic: -7.3	0.76	1.82 mg/mL	Direct titration	sodium edetate

3.5 Precipitation titration

The following table lists the drugs assayed by precipitation titration.

Table 4 List of drugs assayed by precipitation titration

Sl.	Drug Name	pKa	Log P	Solubility	Subtype	Titrant

1	Amidotrizoic Acid Dihydrate	pKa1=1.13(carboxilic acid), pKa2=7.95(amide)	3.3	1.07e-01 g/L	Back titration	Silver Nitrate
2	Benzocaine	2.51	1.86	1310 mg/L at 30°C	Direct titration	Sodium Nitrate
3	Chlordiazepoxide Hydrochloride	4.8	2.44	1 to 5 mg/mL at 66° F	Direct titration	Silver Nitrate

3.6 Redox titration

The following table lists the drugs assayed by redox titration.

Table 5 List of drugs assayed by redox titration

Sl.	Drug Name	pKa	Log P	Solubility	Subtype	Titrant
1	Acetylcysteine	9.52	-0.66	96% soluble in water and ethanol	Direct titration	Iodine
2	Ascorbic Acid	4.7	-1.75	400000mg/L at 40°C	Direct titration	Iodine
3	Hydrous Benzoyl Peroxide	-7.2	3.46	less than 1 mg/mL at 79°C	Direct titration	Sodium Thiosulfate
4	Captopril	pKa1= 3.7, pKa2= 9.8	0.34	1.9×10+5 mg/L at 25°C	Direct titration	Iodine
5	Chlorocresol	9.55	3.1	less than 1 mg/mL at 68° F	Direct titration	Sodium Thiosulfate
6	Chloroxylenol	9.7	3.27	0.03 g/100ml at 15°C, 0.5 g/100ml at 100° C	Direct titration	Sodium Thiosulfate

3.7 HPLC

The following table lists the drugs assayed by HPLC.

Table 6 List of drugs assayed by HPLC

Sl.	Drug Name	pKa	Log P	Solubility	Subtype
1	Acarbose	5.1	-8.08	in water, 1.0×10+6 mg/L, Miscible/ at 25°C	Reverse phase
2	Acetyldigoxin	Strong Acidic: 7.15, Strong basic: -3	2	Insoluble in water	Reverse phase
3	Acitretin	5.1	6.4	In water, 6.61×10^-3 mg/L at 25°C	Reverse phase
		Strong Acidic: 3.99,			Rever

4	Adapalene	Strong basic: -4.8	6.917	4.01e-06 g/L	se phase
5	Alfacalcidol	Strong acidic: 14.39, Strong basic:-2.8	6.68	0.00163 mg/mL	Reverse phase
6	Alfadex	Strong acidic: 11.56, Strong basic: -3.7	-2.4	792mg/mL	Reverse phase
7	Allopurinol	10.2	-1.8	less than 1 mg/mL at 64°F	Reverse phase
8	Alprostadil	4.85	3.2	26.7 mg/L	Reverse phase
9	Altizide	13	1.17	Insoluble in water,	Reverse

				souble in methanol	phase
10	Amlodipine Besilate	Strong Acidic: 19.12, Strong Basic: 9.45	3	0.0074 mg/mL	Reverse phase
11	Amoxicillin Sodium	pKa1=3.2(acid), pKa2=11.7(primary amine)	0.87	in water, 3.43×10+3 mg/L at 25°C	Reverse phase
12	Amoxicillin Trihydrate	Strong Acidic: 3.23, Strong Basic: 7.43	0.87	0.958mg/mL	Reverse phase
13	Ampicillin	2.5	1.35	10100 mg/L at 21°C	Reverse phase
14	Ampicillin Sodium	Strong Acidic: 3.24, Strong Basic: 7.44	1.35	1.01E+004 mg/L	Reverse phase
15	Ampicillin Trihydrate	Strong Acidic: 3.24, Strong Basic: 7.44	1.35	0.605 mg/mL	Reverse phase
16	Atorvastatin Calcium Trihydrate	4.46	6.36	1.12×10-3 mg/L at 25°C	Reverse phase
17	Atracurium Besilate	Strong Acidic: 19.02, Strong Basic: -4.1	3.34	Miscible	Reverse phase
18	Azithromycin	8.74	3.03	In water, 2.37 mg/L at 25°C	Reverse phase
19	Bacampicillin Hydrochloride	Strong Acidic: 11.72, Strong Basic: 7.44	1.17	0.123 mg/mL	Reverse phase
20	Anhydrous Beclometasone Dipropionate	Strong Acidic: 13.85, Strong Basic: -3.3	3.49	2.08e-03 g/L	Reverse phase
21	Beclometasone Dipropionate Monohydrate	Strong Acidic: 13.85, Strong Basic: -3.3	3.69	.00208 mg/mL	Reverse phase
22	Benazepril Hydrochloride	Strong Acidic: 3.53, Strong Basic: 5.36	1.14	>69.1(µg/mL)	Reverse phase
23	Benzathine Benzylpenicillin	2.74	1.83	210 mg/L	Reverse phase
24	Benzylpenicillin Potassium	2.74	1.83	210 mg/mL	Reverse phase
		Strong Acidic: 12.42, Strong Basic: -3.3			Rever

25	Betamethasone		1.138	66.5 mg/L	se phase
26	Betamethasone dipropionate	Strong Acidic: 12.42, Strong Basic: -3.3	4.07	66.5 mg/L	Reverse phase
27	Betamethasone Sodium Phosphate	Strong Acidic: 12.42, Strong Basic: -3.3	1.93	0.0505 mg/mL	Reverse phase
28	Bicalutamide	12	2.5	5 mg/L	Reverse phase
29	Budesonide	Strong Acidic: 13.74, -2.9	1.914	In water, 10.7 mg/L at 25°C	Reverse phase
30	Buserelin	Strong Acidic: 9.49, Strong Basic: 11.85	-3.3	Sparingly soluble in water	Reverse phase
31	Caberogoline	Strong Acidic: 15.25, Strong Basic: 9.32	2.6	insoluble in water	Reverse phase
32	Calcifediol	Strong Acidic: 18.38, Strong Basic: -0.98	6	Insoluble	Reverse phase

33	Anhydrous Calcipotriol	Strong Acidic: 14.39, Strong Basic: -1.6	4.63	0.0135 mg/mL	Reverse phase
34	Calcitriol	Strong Acidic: 14.39, Strong Basic: -1.3	5	insoluble in water	Reverse phase
35	Calcium Folinate	Strong Acidic: 3.47, Strong Basic: 2.81	-3.2	100 mg/mL	Reverse phase
36	Calcium Levofolinate pentahydrate	Strong Acidic: 3.27, Strong Basic: 2.69	-1.1	100 mg/mL	Reverse phase
37	Carbamazepine	15.96, -3.8	2.77	in water, 18mg/L at 25°C	Reverse phase
38	Carbimazole	-3	0.4	3.14e+00 g/L	Reverse phase
39	Carboprost Trometamol	Strong acidic: 4.36, Strong basic: 1.3	3.3	75 mg/mL	Reverse phase
40	Cefaclor	Strong Acidic: 3.03, Strong Basic: 7.44	0.4	10000 mg/L	Reverse phase
41	Cefadroxil Monohydrate	Strong Acidic: 3.45, Strong basic: 7.43	-0.4	1110 mg/L	Reverse phase
42	Cefalexin Monohydrate	5.2, 7.3	0.65	10 mg/mL	Reverse phase
43	Cefalotin Sodium	Strong Acidic: 3.43, Strong basic: -3.2	0	158 mg/L	Reverse phase
44	Cefamandole Nafate	Strong Acidic: 3.1, Strong basic: -1.7	0.61	0.227 mg/mL	Reverse phase
45	Cefapirin Sodium	2.15	-1.15	1030 mg/mL	Reverse phase
46	Cefatrizine Propylene Glycol	Strong Acidic: 2.92, Strong basic: 7.22	-0.28	0.149 mg/mL	Reverse phase
47	Cefazolin Sodium	Strong Acidic: 3.03, Strong basic: 0.26	-0.58	0.487 mg/mL	Reverse phase
48	Cefepime Hydrochloride Monohydrate	Strong Acidic: 3.25, Strong basic: 4.06	-0.37	0.0173 mg/mL	Reverse phase
49	Cefixime	Strong Acidic: 3.45, Strong basic: 2.92	-0.4	55.5 mg/L	Reverse phase
5	Cefoperazone	Strong Acidic: 3.19, Strong basic: -1.7	-0.74	0.286 mg/mL	Reverse

0	Sodium				phase
51	Cefotaxime Sodium	Strong Acidic: 3.18, Strong basic: 4.15	-0.5	68.2 µg/mL	Reverse phase
52	Cefoxitin Sodium	Strong Acidic: 3.59, Strong basic: -3.8	-0.02	0.195 mg/mL	Reverse phase
53	Cefpodoxime proxetil	Strong Acidic: 3.22, Strong basic: 4.16	0.05	0.185 mg/mL	Reverse phase
54	Cefprozil Monohydrate	Strong Acidic: 3.53, Strong basic: 7.43	0.6	55 mg/L	Reverse phase
55	Cefradine	pKa1= 2.6 and pKa2= 7.3	1.5	21300 mg/L	Reverse phase
56	Ceftazidime pentahydrate	Strong Acidic: 2.77, Strong basic: 4.26	-1.6	0.00573 mg/mL	Reverse phase
57	Ceftriaxone Sodium	Strong Acidic: 2.7, Strong basic: 3.36	-1.7	0.105 mg/mL	Reverse phase

58	Cefuroxime Axetil	Strong Acidic: 3.15, Strong basic: -1.1	0.89	107 mg/L at 25°C	Reverse phase
59	Celecoxib	11.1	3.53	4.3 mg/L at 25°C	Reverse phase
60	Chlortalidone	Strong Acidic: 8.76, Strong basic: -2.6	0.85	120 mg/L	Reverse phase
61	Ciclosporin	13.32	1.4	27 µg/mL	Reverse phase
62	Ciprofloxacin Hydrochloride	6.09	-0.57	1.35 mg/mL	Reverse phase
63	Cisplatin		-2.19	1 mg/mL	Reverse phase
64	Cladribine	Strong Acidic: 13.89, Strong basic: 1.33	-0.1	1X10+6 mg/L at 25 °C	Reverse phase
65	Clarithromycin	8.99	3.16	0.33 mg/L	Reverse phase
66	Clindamycin Hydrochloride	7.6	2.16	3.1 mg/mL	Reverse phase
67	Clindamycin Phosphate	7.6	2.16	Soluble in water	Reverse phase
68	Clobetasol Propionate	Strong Acidic: 13.63, Strong basic: -3.4	3.5	0.00413 mg/mL	Reverse phase
69	Cloxacilin Sodium	2.78	2.48	0.0532 mg/mL	Reverse phase
70	Colecalciferol	Strong Acidic: 18.38, Strong Basic: -1.3	7.5	Insoluble in water	Reverse phase
71	Crotamiton	-0.6	2.9	17.7 µg/mL	Reverse phase

3.8 UV Visible Spectroscopy

The following table lists the drugs assayed by UV Visible Spectroscopy.

Table 7 List of drugs assayed by UV Visible Spectroscopy

Sl.	Drug Name	pKa	Log P	Solubility
1	Carmustine	Strong Acidic: 11.96, Strong basic: - 5.3	1.53	4000 mg/L at 25°C
2	Chloramphenicol	Strong Acidic: 7.49, Strong basic: - 2.8	1.14	2500 mg/L
3	Chloramphenicol Palmitate	Strong Acidic: 9.07, Strong basic: - 3.4	7.04	9.51e-05 mg/mL
4	Clobazam	Strong Acidic: 4.07, Strong basic: - 6.7	2.12	188 mg/L
5	Clobetasone Butyrate	Strong Acidic: 12.49, Strong basic: - 3.9	3.76	0.00691 mg/mL
6	Cortisone Acetate	Strong Acidic: 12.6, Strong basic: - 3.8	2.1	20 mg/L

7	Cyanocobalamin	1.84, 8.77	1.897	12.5 mg/mL

3.9 Others

The following table lists the drugs assayed by other methods.

Table 8 List of drugs assayed by other methods

Sl.	Drug Name	pKa	Log P	Solubility	Assay Type
1	Aloxiprin	Strong acidic: 3.41, Strong basic: -7.1	1.19	36.2 mg/mL	UV Visible spectroscopy, gravimetric
2	Amphotericin	Strong Acidic: 3.58, Strong Basic: 9.11	0.8	less than 1 mg/mL at 70°F	Microbiological assay
3	Benorilate	Strong Acidic: 14.66, Strong Basic: -4.4	2.15	6.38e-05 M	gravimetric
4	Bleomycin Sulfate	Strong Acidic: 11.39, Strong Basic: 7.65	-0.41	0.0277 mg/mL	Microbiological assay
5	Colistmethate Sodium	Strong Acidic: -4.3, Strong basic: 6.46	-1.2	4.17 mg/mL	Microbiological assay

Chapter 4

Discussion

From the above tables we can see acid base titration, non-aqueous titration and HPLC are the most used assay techniques for the monographs.

While trying to find out if there is any pattern present or not, in case of aqueous acid base titration it was found that the drugs assayed by this method are mostly highly soluble in water except for a few. Also it is notable that, APIs which are assayed by back titration have comparatively less solubility in water than the directly titrated APIs. In the case of log P value, there is no significant relation between the data. Again, from the pKa values, we can be certain that most of the APIs are highly basic or highly acidic as their pKa values are mostly less than 5 or greater than 9.

Now, drugs assayed by non-aqueous titration are considered, it can be seen from the data that they are mostly insoluble or very less soluble in water. Their solubility may be the main reason behind their assay type. After that, if we come for the log P value, like the acid-base titration, there are no significant patterns. But pKa values can help us to understand that, mostly they are weak acid or weak base because most of the values are between 5-9 range which is applicable for weak acids and bases.

Then, for the drugs assayed by HPLC, unlike the previous two types, they are showing no similarity in case of solubility. In this assay type, APIs are showing completely mix type solubility. Some are very soluble in water, some are sparingly soluble in water, whereas some are insoluble in water. Also, their Log P values are not following any specific pattern. Both positive and negative log P values can be seen significantly in the table. Moreover, their pKa values are also not showing any relation among the data. Assuming from the available data, both strong and weak acids and bases are assayed by HPLC.

These three types of assay account for the majority of assays in the monographs studied which is almost 92%. Only 8% of the APIs are determined by different assay types like complexometric titration, redox titration, precipitation titration, UV-Vis spectroscopy, gravimetric methods and microbiological assay.

In case of complexometric titration, all the compounds have metal. For drugs assayed by complexometric titration method, no standard pattern was noticed in case of solubility, log P and pKa value. The same applies in case of drugs assayed by UV-Visible spectroscopy, redox titration and microbiological assays. Compounds with high solubility and also with insoluble characteristics are assayed by these methods. Also, the Log P and pKa values of the drugs have no specific homogeneity among themselves.

Chapter 5

Conclusion

This study was done to understand whether any specific pattern is present or not in solubility, log p and pKa value among the APIs of BP depending on the assay type. From the study, we have found no significant relation among the data from which we can understand that it is not necessary to have a similar kind of pattern for these kinds of values. Though their assay types can be similar, these properties of the compound can differ largely from one another.

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