FREQUENCY OF ANALYTICAL TECHNIQUES RECOMMENDED BY BRITISH PHARMACOPOEIA (BP)

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Pharmacy

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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.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Frequency of Analytical Techniques Recommended by British Pharmacopoeia (BP)" submitted by Sumaiya Sultana Ema (18346090) of Summer, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Abstract

In this study, the most common assay type for the formulations in British Pharmacopoeia (BP. 2013 edition) monographs was determined. UV-vis spectroscopy, Reverse phase chromatography, Normal phase chromatography, Acid-base titration and non-aqueous titration were found to be the most commonly recommended assay types. Also, some other analytical methods like complexometric titration, redox titration, UV-vis spectroscopy, biological assay have been recommended. Construction of this database will be helpful for educational purposes and future method development.

Keywords: British Pharmacopoeia; Monograph; Assay; Analytical methods.

Dedication

I want to dedicate this to my parents as a simple gratitude as I have done this study with hard work and dedication.

Acknowledgement

The research could not have been completed without the help of numerous individuals who are gratefully acknowledged here. At the very beginning, I would like to thank Almighty Allah for giving me strength and patience to complete the study. I am indebted to my parents who were always there by my side and supported me in my every aspect of life. Then, I would like to express my fullest gratitude to my supervisor, Eshaba Karim, Lecturer, School of Pharmacy, Brac University. She always taught me something new and motivated me to finish this thesis paper. I am thankful to Prof. Dr. Eva Rahman Kabir, Dean, School of Pharmacy and Prof. Dr. Hasina Yasmin, Assistant Dean, School of Pharmacy, Brac University for giving me this opportunity. I am also thankful to academic coordinator and all faculty members of School of Pharmacy.

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List of Acronyms

BP	British Pharmacopoeia
HPLC	High Performance Liquid Chromatography
MHRA	Medicines and Healthcare products Regulatory Agency

Chapter 1

Introduction

1.1 Literature Review

The British Pharmacopoeia (BP) is the United Kingdom's legal pharmacopoeia. It includes both BP (Veterinary), and the European Pharmacopoeia (Ph. Eur.). The UK participates in all phases of the development of the Ph. Eur. monograph and is a member of the EP Commission (World Health Organization (WHO), 2013). The BP significantly contributes to the role of the Medicines and Healthcare products Regulatory Agency (MHRA) in protecting public health by setting a level of quality standards for UK pharmaceutical substances and medicinal products. The BP is used in more than 100 countries and has a large global presence.

Information from the BP was used to conduct this study. There are a few justifications for choosing BP. The most crucial of them is that the BP is regularly updated to reflect new advances in research, changes to regulations, and requirements for pharmaceutical analysis. Surveys are crucial for gathering data and viewpoints from industry specialists, including pharmaceutical manufacturers, analytical labs, medical professionals, and academics. BP makes sure that marketed drugs are effective, safe, and of high quality.

Spectroscopy is recommended in the BP. It is the study of the absorption and emission of light and other radiation by matter. In a manner similar to how a prism divides light into a rainbow of colors, it involves splitting light into its individual wavelengths. In a true form, photographic plates and a prism were used to do traditional spectroscopy. It gives insightful knowledge about the purity, stability, and behavior of pharmaceutical substances by utilizing the interaction of light with matter.

The quantity of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in contrast to a reference or blank sample is measured by the analytical technique known as UV-vis spectroscopy. The energy of light has a fixed value that is inversely proportional to its wavelength (Spectroscopy, n.d.). As a result, shorter light wavelengths carry more energy while longer ones carry less. In order to promote electrons in a substance to a higher energy state, a certain amount of energy is required, that can be observed as an absorption. The UV range typically spans 190nm to 400nm whereas, the visible spectrum is between 400nm to 800 nm (Kumar, 2006). Light source, monochromator, sample cuvette, diffraction grating and detectors are the main instrumentation used for UV-vis spectroscopy. As a light source, deuterium lamps, Xenon lamps, and LED lamps are used. However, since LED lamps only produce light at a single wavelength, a monochromator is not necessary (Patel et al., 2022). Detectors are used to detect and transform a reflected or transmitted light from sample into signal. Photomultiplier tube is a most common detector used for this technique. To measure the ultraviolet range, reusable quartz cuvettes are required because most of the time, glass and plastics absorb the light. In the study of analytical chemistry, UV-visible spectroscopy is frequently used, particularly when performing a quantitative examination of a particular analyte. For instance, UV-visible spectroscopy may be used to quantitatively analyze conjugated organic molecules, to check the purity of biological compounds like- nucleic acid, DNA or RNA (Mettler Toledo GmbH, 2021).

Like other techniques, spectroscopy has both advantages and limitations. Some of these advantages include the ability to reuse or continue processing or analysis of the sample due to the non-destructive nature of the procedure. Quick measurement allows for simple experimental techniques. This technique is very easy to operate and requires little instruction for the user before use. Since the instrument is usually affordable to purchase, many laboratories can use it. In contrast the limitations can include the fact that in a practical

instrument, wavelength selectors are not perfect and a little amount of light from a wide wavelength range may potentially lead to substantial measurement failures. The other sources of light include the environment and a loosely fitting instrument compartment. Second, bubbles in the cuvette or sample will scatter light, producing results that are irreproducible and inaccurate. Each chemical species should be removed from the sample and evaluated separately in order to conduct a proper quantitative analysis. Any component of the instrument that is misaligned or positioned incorrectly could result in unreliable results (PhD, 2021). Therefore, it is important that every part of the instrument is oriented in the same manner.

Chromatography is a separation technique used to separate compounds from mixture. The mixture's components are distributed in a liquid solution known as the mobile phase, which binds them together as they pass through a structure containing a different substance called as the stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid. Chromatography is one of several separation techniques defined as differential migration from a narrow initial zone (Sayed, 2021).

For the separation and analysis of a variety of chemicals, reverse phase chromatography (RPC) is a commonly used method in liquid chromatography. Analytes are separated based on their hydrophobicity using the idea of differential affinity between a nonpolar stationary phase and a polar mobile phase. Hydrophobic interactions between the analyte molecules and the stationary phase are the basis for reverse phase chromatography. Typically, the stationary phase is a column of nonpolar material, such as bonded or chained hydrocarbons on a solid support. In contrast, the mobile phase is made up of a polar solvent, usually water or a combination of water and an organic solvent like acetonitrile or methanol. While more polar molecules elute quicker, analytes with higher hydrophobicity spend more time interacting with the stationary phase and elute more slowly. An injection port or autosampler for introducing samples, a column for separation, a detector, and a data analysis system are the standard components of a

reverse phase chromatography system (Merck, 2021). The solvent supply system makes sure that the mobile phase flows continuously through the column. As the analyte elutes from the column, the UV/Vis detector often measures the concentration of the analyte. For extra information, other detectors can also be used, including mass spectrometers.

The mobile phase in reverse phase chromatography, the ratio of water to an organic solvent is often changed to obtain the desired separation. Methanol, acetonitrile or a mixture of the two are typical organic solvents utilized in this context. The selectivity and resolution of the separation are impacted by the composition of the mobile phase. And the stationary phase in reverse phase chromatography is made up of hydrophobic substances that are packed into a column or coated or chemically bound to the surface of a solid support. Commonly utilized silica-based compounds include those with hydrophobic functional groups like C18 (octadecyl) or C8 (octyl) chains. To maximize separation for a given analyte, hydrophobic chains may be altered in length and type (Merck, 2021). The stationary phase is typically a polar material, such as silica gel or alumina, which can be coated onto a solid support or packed into a column. The mobile phase consists of a less polar organic solvent or a mixture of organic solvents.

A common chromatographic method for the separation and analysis of both polar and highly polar substances is normal phase chromatography (NPC). The separation of analytes based on their polarity and interaction with the stationary phase is achieved using the idea of differential affinity between a polar stationary phase and a less polar mobile phase. The polarity differences between the analytes and the stationary phase are the basis of normal phase chromatography. A polar substance, such silica gel or alumina, which may be deposited onto a solid support or crammed into a column, serves as the stationary phase in most reactions. A less polar organic solvent or a combination of organic solvents make up the mobile phase (Sayed, 2021). Differential partitioning results from the analytes' distinct interactions with the polar stationary phase and the less polar mobile phase, which causes separation. An injection port or

autosampler for introducing samples, a column filled with the polar stationary phase, a detector for keeping track of the separating compounds, and a data analysis system make up the instrumentation of a typical normal phase chromatographic system. For chemical detection and quantification, a number of detectors may be utilized, including UV/Vis, fluorescence, and mass spectrometry.

A less polar organic solvent or a combination of organic solvents makes up the mobile phase in normal phase chromatography. Hexane, heptane, and ethyl acetate are examples of frequently used organic solvents, as are their mixtures with other polar solvents as dichloromethane or tetrahydrofuran. The polarity of the analyte and the preferred separation conditions influence the selection of the mobile phase composition. A polar substance, such silica gel or alumina, is often used as the stationary phase in normal phase chromatography. Due to its availability, chemical stability and wide range of applications, silica gel is the most often used stationary phase. To improve selectivity and regulate the retention of certain analytes, functional groups may be added to the surface of the stationary phase.

The group of analytical separation techniques used for analyzing volatile compounds in the gas phase is known as gas chromatography. By dividing the sample between two phases-a stationary phase and a mobile phase-in gas chromatography, the components of a sample are dissolved in a solvent and vaporized to separate the analytes. The analyte molecules are transported through the heated column by the mobile phase, which is a chemically inert gas. The stationary phase is a high-boiling liquid or solid coated on a solid support material, typically packed into a column or coated on the walls of a capillary column. The mobile phase is an inert carrier gas, such as helium or nitrogen, which transports the sample through the column (Jackie & Shimadzu, 2020). There are various important parts that make up a gas chromatograph. An injection port, a column, carrier gas flow control equipment, ovens and heaters to regulate the temperatures of the injection port and the column, an integrator chart

recorder, and a detector make up a conventional gas chromatograph. Either a sample introduction mechanism that is automated or an injection port are used to introduce the sample into the instrument. The mobile phase carries the vaporized sample into the column. The separation of analytes based on their partitioning behavior is provided by the column, which is housed in an oven. After being separated, the components are brought to a detector, which produces signals proportional to the chemical concentrations. Flame ionization detectors (FID), thermal conductivity detectors (TCD), electron capture detectors (ECD), and mass spectrometers (MS) are often used detectors in GC (Kaur & Sharma, 2018).

Titration is a method used for calculating a solution's concentration. Once the reaction approaches neutralization, which is often indicated by a color change, it involves progressively adding known-concentration titrant to unknown-concentration analytical solutions.

A quantitative analytical technique known as an acid-base titration involves neutralizing an acid or base with a standard solution of known concentration in order to determine its concentration. It involves using a pipette and burette to determine the acid or base concentration. A colorant used as an indication is one that is added to a solution to change its color. It disintegrates in the sample solution and acts as an easy-to-use titration indicator. The pH range of the titration, the intended endpoint, and the indicator's sensitivity are taken into consideration while selecting an indicator for an acid-base titration. A few examples of frequently used indicators include bromothymol blue, phenolphthalein, methyl red, thymol blue, and methyl orange. Due to its limited pH range and rapid color shift at the endpoint, phenolphthalein is an ideal indicator for a weak acid-strong base titration.

It is necessary to utilize non-aqueous titration because water interacts with other dissolved weak acids and bases for proton donation or acceptance because it can act as both a weak base and an acid. Non-aqueous titration is a very useful procedure because it fulfills two criteria that

may be used to titrate extremely weak acids or bases and it offers a solvent that might dissolve organic compounds. Color change at the end point varies because changing color depend on the nature of the titrant (Rastogi, 2018). One can determine the purity of the tests using non-aqueous titration. It is used to determine concentration expression, to recognize steroids, phenobarbitone, diuretics, and hydrophobic compounds. Non-aqueous titration is also used to evaluate adrenergic and antitubercular drug compositions.

Reduction-Oxidation is referred to as redox. In these titrations, an oxidizing agent is titrated with a reducing agent, or vice versa. For the reaction to complete with a sharp end point, the oxidizing and reducing capacities of these agents must be substantially different. Oxidation involves electron loss whereas reduction involves electron gain (Marie, 2015). Through the use of a redox interaction between the analyte and the titrant, redox titration is an analytical technique used for determining the concentration of an analyte. A potentiometer or redox indicator is widely used for the redox titration. The Redox titration procedure is based on an oxidation-reduction reaction between the titrant and analyte. It is also one of the methods to determine concentration that is widely used.

The process, known as complexometric titration, involves titrating metal ions with a complexing agent or chelating agent (ligand). This approach is an analytical use of a complexation reaction. In this procedure, a simple ion is converted into a complex ion, and the equivalence point is identified either electrometrically or by the use of metal indicators. In complexometric titration which is a type of volumetric titration, the development of a colored complex is utilized to denote the titration's end point. A metal ion reacts with an anion, a neutral molecule, or rarely a positive ion to create the complexes. Complexometric titrations can be used to identify a mixture of different metal ions in solution (Husain, 2017).

The formation of hardly soluble salts of the reagent and the sample serves as the foundation for precipitation titration. In a potentiometric titration, several ions generate products that are poorly soluble with various solubility products with the reagent exhibit many equivalence points. In a precipitation titration, turbidity either develops during the titration and peaks at the conclusion of the reaction, or its presence signals the conclusion of the reaction. Both times, the turbidity scatters the light that the sensor emits, changing the intensity on the detector as a result (Reining, 2018)

1.2 Aim and Objective

The aim of this study is to give an idea about how frequently the different types of assays are used and recommended by the British Pharmacopoeia. It gives an overall review about the analytical techniques. It also includes comparison between the assay and different sub-assay types, their strengths and limitations. The provided information can be used for further study purpose.

Chapter 2

Method

How the assessment was done and assays were identified are given below with the help of a flow chart:

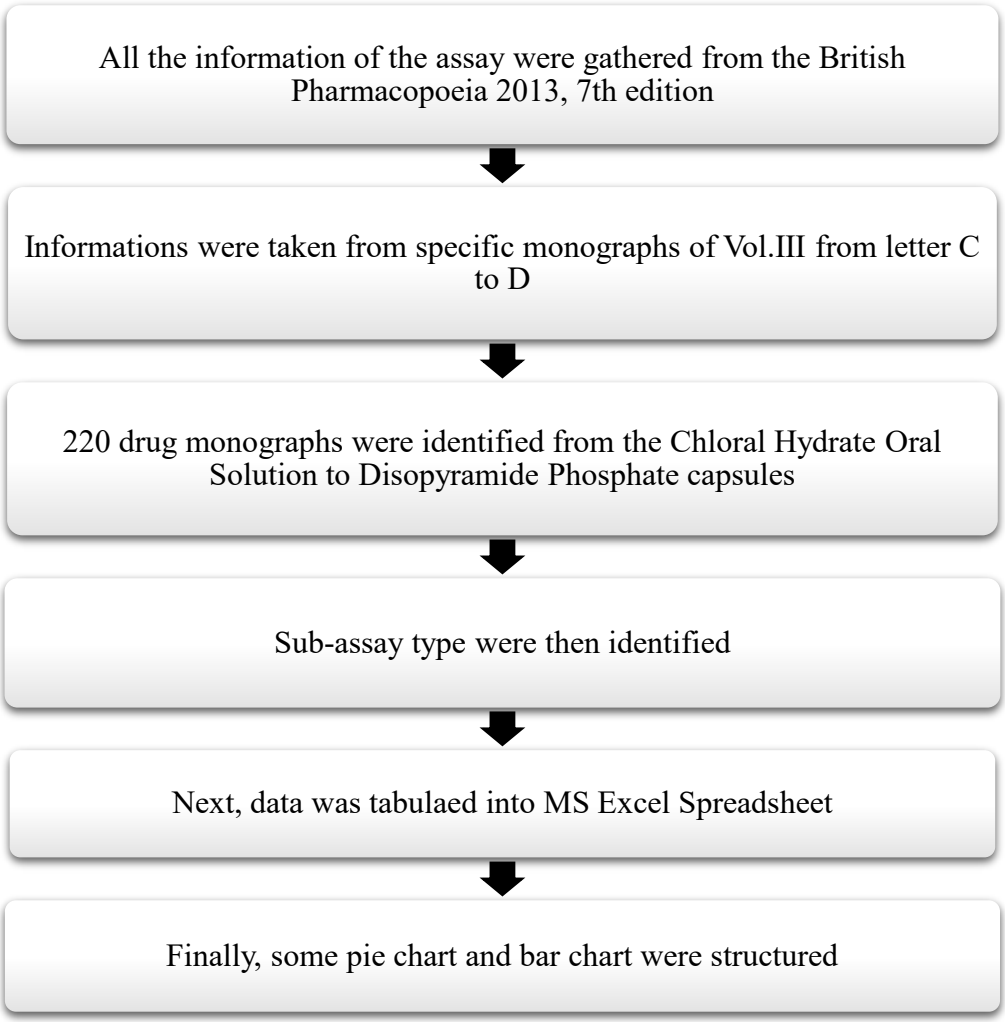

Figure 1: Methodology

Chapter 3

Result and Discussion

From the following database table, results were extracted and discussion were done with all the comparison, advantages and limitations. This table can be helpful for further particular area of study.

Drug	Dosage form	Assay type	Assay subtype
Chloral Hydrate Oral Solution	solution	titrimetric	Precipitation
Chlorambucil Tablets	tablet	chromatographic	reverse phase HPLC
Chloramphenicol Capsules	capsule	chromatographic	reverse phase HPLC
Chloramphenicol Ear Drops	ear drops	chromatographic	reverse phase HPLC
Chloramphenicol Eye Drops	eye drops	chromatographic	reverse phase HPLC
Chloramphenicol Eye Ointment	ointment	chromatographic	reverse phase HPLC
Chloramphenicol Sodium Succinate Injection	injection/infusion	spectroscopic	UV
Chlordiazepoxide Capsules	capsule	spectroscopic	UV

Chlordiazepoxide Hydrochloride Tablets	tablet	spectroscopic	UV
Chlorhexidine Gluconate Eye Drops	eye drops	chromatographic	reverse phase HPLC
Chlorhexidine Gluconate gel	gel	chromatographic	reverse phase HPLC
Chlorhexidine Irrigation Solution	solution	chromatographic	reverse phase HPLC
Chlorhexidine Mouthwash	solution	chromatographic	reverse phase HPLC
Chloroform Spirit	spirit/elixir/linctus	chromatographic	gas chromatography
Chloroform and Morphine Tinctire	solution	spectroscopic	UV
Double Strength Chloroform Water	solution	assay not mentioned	
Chlorquine Phosphate Tablets	tablet	titrimetric	non aqueous titration
Chlorquine Sulfate Tablets	tablet	titrimetric	non aqueous titration
Chloroxylenol Solution	solution	chromatographic	gas chromatography

Chlorphenamine Injection	injection/infusion	spectroscopic	UV
Chlorphenamine Oral Solution	solution	chromatographic	gas chromatography
Chlorphenamine Tablets	tablet	spectroscopic	UV
Chlorpromazine Injection	injection/infusion	spectroscopic	UV
Chlorpromazine Oral Solution	solution	spectroscopic	UV
Chlorpromazine Suppositories	suppository	spectroscopic	UV
Chlorpromazine Tablets	tablet	spectroscopic	UV
Chlorpropamide Tablets	tablet	spectroscopic	UV
Chlortalidone Tablets	tablet	spectroscopic	UV
Chlortetracycline Eye Ointment	ointment	chromatographic	reverse phase HPLC
Chlortetracycline Ointment	ointment	chromatographic	reverse phase HPLC
Choline Salicylate Ear Drops	ear drops	titrimetric	non aqueous titration
Choline Salicylate Oromucosal Gel	gel	titrimetric	non aqueous titration

Choline Theophyllinate Tablets	tablet	spectroscopic	UV
Chorionic Gonadotrophin Injection	injection/infusion	biological	
Ciclosporin Eye Drops	eye drops	chromatographic	reverse phase HPLC
Cimetidine Injection	injection/infusion	spectroscopic	UV
Cimetidine Oral Solution	solution	chromatographic	reverse phase HPLC
Cimetidine Oral Suspension	suspension	chromatographic	reverse phase HPLC
Cimetidine Tablets	tablet	spectroscopic	UV
Ciprofloxacin Infusion	injection/infusion	chromatographic	reverse phase HPLC
Ciprofloxacin Tablets	tablet	chromatographic	reverse phase HPLC
Cisplatin Injection	injection/infusion	chromatographic	reverse phase HPLC
Clarithromycin for Infusion	injection/infusion	chromatographic	reverse phase HPLC
Clarithromycin Tablets	tablet	chromatographic	reverse phase HPLC

Prolonged-release Clarithromycin Tablets	tablet	chromatographic	reverse phase HPLC
Clemastine Oral Solution	solution	chromatographic	reverse phase HPLC
Clemastine Tablets	tablet	chromatographic	reverse phase HPLC
Clindamycin Capsules	capsule	chromatographic	reverse phase HPLC
Clindamycin Injection	injection/infusion	chromatographic	reverse phase HPLC
Clobazam Oral Suspension	suspension	chromatographic	reverse phase HPLC
Clobazam Tablets	tablet	chromatographic	reverse phase HPLC
Clobetasol Cream	cream	chromatographic	reverse phase HPLC
Clobetasol Ointment	ointment	chromatographic	reverse phase HPLC
Clobetasone Cream	cream	chromatographic	reverse phase HPLC
Clobetasone Ointment	ointment	chromatographic	reverse phase HPLC

Clofazimine Capsules	capsule	chromatographic	reverse phase HPLC
Clofibrate Capsules	capsule	assay not mentioned	
Clomethiazole Capsules	capsule	titrimetric	non aqueous titration
Clomethiazole Intravenous Infusion	injection/infusion	chromatographic	reverse phase HPLC
Clomethiazole Oral Solution	solution	chromatographic	reverse phase HPLC
Clomifene Tablets	tablet	spectroscopic	UV
Clomipramine Capsules	capsule	spectroscopic	UV
Clonazepam Injection	injection/infusion	spectroscopic	UV
Clonazepam Oral Suspension	suspension	chromatographic	reverse phase HPLC
Clonazepam Tablets	tablet	chromatographic	reverse phase HPLC
Clonidine Injection	injection/infusion	spectroscopic	UV
Clonidine Tablets	tablet	spectroscopic	UV
Clotrimazole Cream	cream	chromatographic	reverse phase HPLC

Clotrimazole Pessaries	pessary	chromatographic	reverse phase HPLC
Clozapine Oral Suspension	suspension	chromatographic	reverse phase HPLC
Co-amilofruse Tablets	tablet	chromatographic	reverse phase HPLC
Co-amilozide Oral Solution	solution	chromatographic	reverse phase HPLC
Co-amilozide Tablets	tablet	chromatographic	reverse phase HPLC
Co-amoxiclav Injection	injection/infusion	chromatographic	reverse phase HPLC
Co-amoxiclav Oral Suspension	suspension	chromatographic	reverse phase HPLC
Co-amoxiclav Tablets	tablet	chromatographic	reverse phase HPLC
Dispersible Co-amoxiclav Tablets	tablet	chromatographic	reverse phase HPLC
Co-beneldopa Capsules	capsule	chromatographic	reverse phase HPLC
Dispersible Co-beneldopa Tablets	tablet	chromatographic	reverse phase HPLC

Cocaine Eye Drop	eye drops	chromatographic	reverse phase HPLC
Cocaine Paste	paste	chromatographic	reverse phase HPLC
Co-cereldopa Tablets	tablet	chromatographic	reverse phase HPLC
Co-codamol Capsules	capsule	chromatographic	reverse phase HPLC
Co-codamol Tablets (Codeine)	tablet	chromatographic	reverse phase HPLC
Co-codamol Tablets (Paracetamol)	tablet	chromatographic	reverse phase HPLC
Effervescent Co-codamol Tablets (Codeine)	effervescent tablet	chromatographic	reverse phase HPLC
Effervescent Co-codamol Tablets (Paracetamol)	effervescent tablet	chromatographic	reverse phase HPLC
Co-danthrusate Capsules (Dantron)	capsule	spectroscopic	UV
Codeine Linctus	solution	chromatographic	reverse phase HPLC
Paediatric Codeine Linctus	solution	chromatographic	reverse phase HPLC

Codeine Phosphate Injection	injection/infusion	chromatographic	reverse phase HPLC
Codeine Phosphate Oral Solution	solution	chromatographic	reverse phase HPLC
Codergocrine Tablets	tablet	chromatographic	reverse phase HPLC
Co-dydramol Tablets (Dihydrocodeine tartrate	tablet	chromatographic	reverse phase HPLC
Co-dydramol Tablets (Paracetamol)	tablet	chromatographic	reverse phase HPLC
Co-fluampicil Capsules	capsule	chromatographic	reverse phase HPLC
Co-fluampicil Oral Suspension (Ampicillin)	suspension	spectroscopic	UV
Co-fluampicil Oral Suspension	suspension	spectroscopic	UV
Colchicine Tablets	tablet	spectroscopic	UV
Colecalciferol Injection	injection/infusion	spectroscopic	UV
Colecalciferol Tablets	tablet	spectroscopic	UV
Colestipol Granules	powder	assay not mentioned	
Colestyramine Oral Powder	powder	spectroscopic	UV

Colistimethate Injection	injection/infusion	biological	
Colistimethate Nebuliser Solution	solution	biological	
Colistin Tablets	tablet	biological	
Flexible Collodion	solution	assay not mentioned	
Co-magaldrox Oral Suspension (Al2O3)	suspension	titrimetric	complexometric titration
Co-magaldrox Oral Suspension (Mg(OH)2)	suspension	titrimetric	complexometric titration
Co-magaldrox Tablets (Al2O3)	tablet	titrimetric	complexometric titration
Co-magaldrox Tablets (Mg(OH)2)	tablet	titrimetric	complexometric titration
Co-proxamol Tablets (dextropropoxyphene hydrochloride)	tablet	chromatographic	reverse phase HPLC
Co-proxamol Tablets (Paracetamol)	tablet	spectroscopic	UV
Cortisone Tablets	tablet	chromatographic	reverse phase HPLC
Co-tenidone Tablets	tablet	chromatographic	reverse phase HPLC

Co-triamterzide Tablets	tablet	chromatographic	reverse phase HPLC
Co-trimoxazole Infusion (trimethoprim)	injection/infusion	spectroscopic	UV
Co-trimoxazole Infusion (sulfamethoxazole)	injection/infusion	titrimetric	Precipitation
Co-trimoxazole Oral Suspension (trimethoprim)	suspension	spectroscopic	UV
Co-trimoxazole Oral Suspension (sulfamethoxazole)	suspension	spectroscopic	UV
Paediatric Co-trimoxazole Oral Suspension (trimethoprim)	suspension	spectroscopic	UV
Paediatric Co-trimoxazole Oral Suspension (sulfamethoxazole)	suspension	spectroscopic	UV
Co-trimoxazole Tablets (trimethoprim)	tablet	spectroscopic	UV
Co-trimoxazole Tablets (sulfamethoxazole)	tablet	titrimetric	Precipitation

Dispersible Co-trimoxazole Tablets (trimethoprim)	tablet	spectroscopic	UV
Dispersible Co-trimoxazole Tablets (sulfamethoxazole)	tablet	titrimetric	Precipitation
Paediatric Co-trimoxazole Tablets (trimethoprim)	tablet	spectroscopic	UV
Paediatric Co-trimoxazole Tablets (sulfamethoxazole)	tablet	titrimetric	Precipitation
Crotamiton Cream	cream	chromatographic	normal phase HPLC
Crotamiton Lotion	lotion	chromatographic	normal phase HPLC
Cyanocobalamin Tablets	tablet	chromatographic	reverse phase HPLC
Cyclizine Injection	injection/infusion	spectroscopic	UV
Cyclizine Tablets	tablet	spectroscopic	UV
Cyclopenthiazide Tablets	tablet	spectroscopic	UV
Cyclopentolate Eye Drops	eye drops	chromatographic	reverse phase HPLC
Cyclophosphamide Injection	injection/infusion	titrimetric	Precipitation

Cyclophosphamide Oral Solution	solution	chromatographic	reverse phase HPLC
Cyclophosphamide Tablets	tablet	titrimetric	Precipitation
Cyproheptadine Tablets	tablet	spectroscopic	UV
Cyproterone Tablets	tablet	chromatographic	reverse phase HPLC
Cytarabine Injection	injection/infusion	chromatographic	reverse phase HPLC
Dacarbazine Injection	injection/infusion	spectroscopic	UV
Dalteparin Sodium Injection	injection/infusion	biological	
Dantrolene Oral Suspension	suspension	chromatographic	normal phase HPLC
Dapsone Tablets	tablet	titrimetric	Precipitation
Demeclocycline Capsules	capsule	chromatographic	reverse phase HPLC
Desferrioxamine Injection	injection/infusion	titrimetric	redox titration
Desipramine Tablets	tablet	spectroscopic	UV
Desmopressin Injection	injection/infusion	chromatographic	reverse phase HPLC

Desmopressin Intranasal Solution	solution	chromatographic	reverse phase HPLC
Desmopressin Tablets	tablet	chromatographic	reverse phase HPLC
Desogestrel Tablets	tablet	chromatographic	reverse phase HPLC
Dexamethasone Eye Drops, Suspension	suspension	chromatographic	reverse phase HPLC
Dexamethasone Tablets	tablet	chromatographic	reverse phase HPLC
Dexamethasone and Neomycin Ear Spray (Dexamethasone)	spray	chromatographic	reverse phase HPLC
Dexamethasone and Neomycin Ear Spray (Neomycin)	spray	biological	
Dexamethasone Sodium Phosphate Ear Drops, Solution	ear drops	chromatographic	reverse phase HPLC
Dextramethasone Sodium Phosphate Injection	injection/infusion	chromatographic	reverse phase HPLC

Dextramethasone Sodium Phosphate Oral Solution	solution	chromatographic	reverse phase HPLC
Dextran 40 Infusion	injection/infusion	polarimetry	optical rotation
Dextran 70 Infusion	injection/infusion	polarimetry	optical rotation
Dextromoramide Tablets	tablet	titrimetric	non aqueous titration
Dextropropoxyphene Capsules	capsule	titrimetric	non aqueous titration
Diamorphine Injection	injection/infusion	spectroscopic	UV
Diazepam Injection	injection/infusion	spectroscopic	UV
Diazepam Oral Solution	solution	spectroscopic	UV
Diazepam Rectal Solution	solution	spectroscopic	UV
Diazepam Tablets	tablet	spectroscopic	UV
Diazoxide Injection	injection/infusion	spectroscopic	UV
Diazoxide Tablets	tablet	spectroscopic	UV
Dichlorophen Tablets	tablet	spectroscopic	UV
Prolonged-release Diclofenac Capsules	capsule	spectroscopic	UV
Diclofenac Gel	gel	chromatographic	reverse phase HPLC

Gastro-resistant Diclofenac Tablets	tablet	chromatographic	reverse phase HPLC
Prolonged-release Diclofenac Tablets	tablet	chromatographic	reverse phase HPLC
Dicycloverine Oral Solution	solution	titrimetric	redox titration
Dicycloverine Tablets	tablet	titrimetric	redox titration
Diethylamine Salicylate Cream	cream	spectroscopic	UV
Diethylcarbamazine Tablets	tablet	chromatographic	reverse phase HPLC
Diethylstilbestrol Pessaries	pessary	spectroscopic	UV
Diethylstilbestrol Tablets	tablet	spectroscopic	UV
Diflucortolone Cream	cream	chromatographic	reverse phase HPLC
Diflucortolone Oily Cream	cream	chromatographic	reverse phase HPLC
Diflucortolone Ointment	ointment	chromatographic	reverse phase HPLC
Diflunisal Tablets	tablet	spectroscopic	UV
Digitoxin Tablets	tablet	spectroscopic	UV

Digoxin Injection	injection/infusion	spectroscopic	UV
Paediatric Digoxin Injection	injection/infusion	spectroscopic	UV
Paediatric Digoxin Oral Solution	solution	spectroscopic	UV
Digoxin Tablets	tablet	spectroscopic	UV
Dihydrocodeine Injection	injection/infusion	spectroscopic	UV
Dihydrocodeine Oral Solution	solution	chromatographic	reverse phase HPLC
Dihydrocodeine Tablets	tablet	titrimetric	non aqueous titration
Diloxanide Tablets	tablet	spectroscopic	UV
Prolonged-release Diltiazem Tablets	tablet	chromatographic	reverse phase HPLC
Dimercaprol Injection	injection/infusion	titrimetric	redox titration
Dinoprostone Oral Solution	solution	chromatographic	reverse phase HPLC
Diphenhydramine Tablets	tablet	chromatographic	reverse phase HPLC
Dipipanone and cyclizine Tablets	tablet	chromatographic	gas chromatography

Dipivefrine Eye Drops	eye drops	chromatographic	reverse phase HPLC
Prolonged-release Dipyridamole Capsules	capsule	chromatographic	reverse phase HPLC
Dipyridamole Infusion	injection/infusion	chromatographic	reverse phase HPLC
Dipyridamole Oral Suspension	suspension	chromatographic	reverse phase HPLC
Dipyridamole Tablets	tablet	chromatographic	reverse phase HPLC
Disodium Edetate Eye Drops	eye drops	chromatographic	reverse phase HPLC
Disopyramide Capsules	capsule	spectroscopic	UV
Disopyramide Phosphate Capsules	capsule	spectroscopic	UV

Table 1: Database Table

From the monograph studied, the Chromatographic technique is almost 49% of the total which was mostly recommended. Recommendation of Spectroscopic and Titrimetric technique stands for 29% and 16% respectively. Moreover, Biological stands for 3% and Polarimetry stands for only 1% whereas, the remaining 2% of monographs are not mentioned (Figure 2).

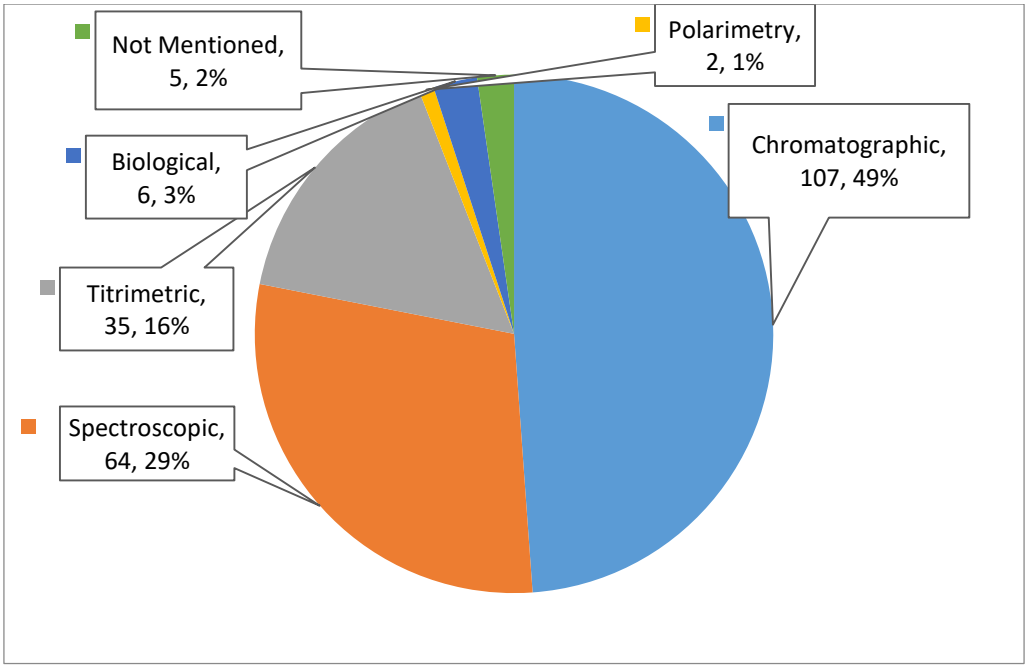

Figure 2: Assay Types

As it has been already mentioned that among all the techniques, Chromatography has been recommended the most which is only because of its own advantage. The advantages are like- Chromatography promotes correct methods to perform separation, analysis, and purification. This technique is widely used for its accurate characteristics. In chromatography, very small amount of sample is required. Each component of a mixture, can be separated and collected through chromatography. Chromatography can analyze a wide range of materials, including tissue extracts, air and water samples, pesticides, plastics, food particles, and drugs among others. Chromatography can be used to separate highly complex mixtures.

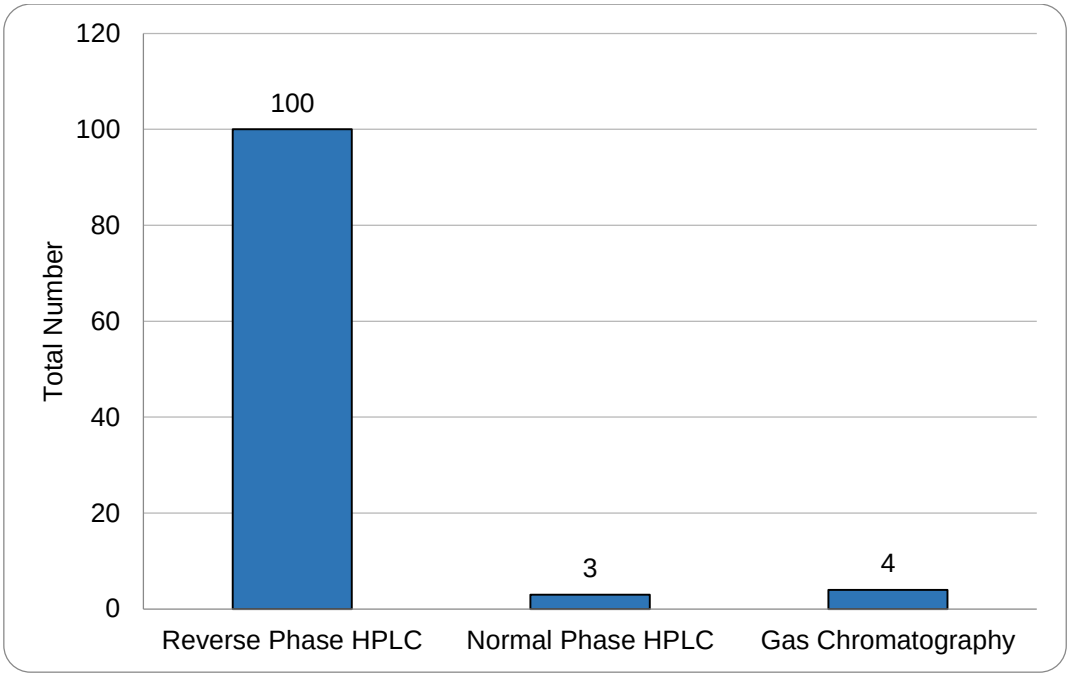

Figure 3: Chromatographic Techniques

From the recommended chromatographic technique Figure 3, reverse phase HPLC has been used for 100 times, Gas chromatography for 4 times and normal phase HPLC for 3 times. Reverse phase chromatography has been recommended for several times because it is better than other HPLC method. With the utilization of a hydrophobic stationary phase, reverse phase can evaluate samples that contain polar (hydrophilic), non-polar (hydrophobic), ionic, and ionizable chemical compounds. The following method employs nontoxic solvents, offers a technique for removing impurities and mobile phase additives, and delivers rapid sample recovery with little solvent evaporation. It also offers improved solubility for polar analytes. It has lower costs in comparison to other chromatographic methods. It contains a less hazardous solvent, and because of this, it harms the environment considerably less. This technique has the ability to improve gradient separation efficiency. For precise outcomes, reverse phase chromatography entails a smaller sample size.

High resolution, great repeatability, broad application for a variety of chemicals, and compatibility with various detection techniques are some benefits of reverse phase chromatography. It is suitable for the study of hydrophobic substances and enables the separation and characterization of complicated mixtures. With its broad spectrum of separation capabilities, reverse phase chromatography is able to separate a variety of substances, including polar in nature nonpolar, hydrophobic, and hydrophilic analytes. It may thus be used with a broad range of sample variations. Aqueous mobile phases, as opposed to fully organic mobile phases, are often more affordable and ecologically harmless and are compatible with this chromatographic process. Mobile phases based on water make it easier to analyze polar and hydrophilic substances. With this method, you may choose from a wide variety of stationary phase columns with different pore diameters, surface chemistries, and hydrophobicities. This enables personalized separations depending on particular analyte properties, increasing resolution and selectivity. Due to the homogeneous particle packing in the column, it offers good separation efficiency. This produces sharp, well-defined peaks that are narrow and allow for the identification and measurement of small components in complicated mixtures. Because of its great detection sensitivity, it is possible to analyze chemicals at the trace level. It is also compatible with a number of detection methods, including UV/Vis, fluorescence, electrochemistry, and mass spectrometry, giving researchers a variety of choices for identifying and quantifying compounds. RPC techniques are often reliable and repeatable, providing consistent outcomes using various equipment and labs. For technique transfer, method validation, and regular analysis in quality control labs, this dependability is essential. Scaling up this approach from analytical to preparative or industrial sizes is simple. RPC may therefore be used for both small- and large- scale research and purifying procedures.

Although reverse phase chromatography is a diverse method, it has several drawbacks. For strongly polar or ionic substances with weak interactions with the stationary phase, it may not

be appropriate. Compared to normal phase chromatography (NPC), it is less successful in separating highly polar substances. Low retention of highly polar molecules on the nonpolar stationary phase may result in poor separation and resolution. Moreover, substances with comparable hydrophobicity may elute together, resulting in co-elution and less separation. Small polar molecules have a significant contact with the aqueous mobile phase, which makes RPC difficult to retain them. RPC columns can experience stability issues, especially when exposed to harsh or extreme conditions. The stationary phase may degrade over time, leading to changes in selectivity and decreased column efficiency. Additionally, reverse phase is a time-consuming extensive procedure. It's repeatability and resolving power is poor. This method typically operates in the mid to low retention time range. Compounds with extremely high or low retention times may be challenging to separate effectively using RPC alone. In such cases, alternative chromatographic techniques may be necessary. It's important to note that the disadvantages mentioned above are not universally applicable and may vary depending on the specific experimental setup, analyte characteristics, and application requirements. Careful method development, optimization, and selection of appropriate columns and conditions can help mitigate these limitations and improve the performance of reverse phase chromatography (Merck, 2021).

On the other hand, normal phase chromatography is not suitable for separation of ionic and polar compounds. Extremely hydrophilic or ionic chemicals frequently exhibit low solubility in the organic mobile phase that are frequently utilized in normal phase and tend to get too strongly retained on polar adsorbents.

Normal phase chromatography offers several advantages. It provides excellent separation and resolution for polar compounds that may not be well-suited for other chromatographic techniques. It is excellent in separating polar and extremely polar substances. For polar analytes such as organic acids, sugars, amino acids, peptides, and water-soluble vitamins, it gives good

retention and resolution. For the investigation of substances that have powerful polar interactions with the stationary phase, it is very helpful. It enables the study of a variety of polar molecules and is particularly helpful for the separation of isomeric and structurally related compounds. Numerous analytes including polar, moderately polar, and even nonpolar substances, may be handled by NPC. It is appropriate for a variety of sectors, including the pharmaceuticals industry, since it provides adaptability for broad sample types and applications. Polar solvent contained mobile phases are may be more affordable and environment friendly than the organic mobile phases used in other chromatographic modes which are compatible with normal phase chromatography.

On the other hand, there are several drawbacks with normal phase chromatography to be aware of. Since their interactions with the polar stationary phase are weaker, it is less effective for the separation of nonpolar or moderately polar substances. Short retention durations and poor separation may be characteristics of highly nonpolar molecules. Additionally, adequate handling and storage are necessary since the polar stationary phase might be susceptible to moisture and hydrolysis. Silica gel, one of the polar stationary phases used in NPC, is susceptible to moisture and may hydrolyze in the presence of water. As a result, the performance of the column may decline and its selectivity may alter. Due to the limitations on the stability of the stationary phase, the pH range of the mobile phase in normal phase chromatography is constrained. Extreme pH levels may degrade or permanently transform the stationary phase. The regeneration procedure may be time-consuming and may not completely restore the column's former performance, even if certain normal phase columns can be regenerated and reused. Costs may rise as a result, particularly if repeated column replacements are required. When selecting the best chromatographic method for a given study, it's crucial to take these benefits and drawbacks into account. Depending on the analytes, sample matrices,

required separations, and resources available, normal phase chromatography or a different technique is adopted.

Additionally, because to its limitations, gas chromatography was only used four times during the whole chromatographic method. Only volatile samples are suitable for gas chromatography. For samples that are thermally labile, this method is unsuitable. Samples should be selected for this approach in a manner that prevents them from becoming soluble and from reacting with the column. Carrier gas used should be of pure form, for example, pure nitrogen gas. It is least sensitive to compounds whose molecules have negligible affinity for electrons. Gas chromatography offers several advantages, including high separation efficiency, wide range of analyte compatibility, excellent sensitivity, and rapid analysis times. It allows for the identification and quantification of complex mixtures and can handle both small and large sample sizes. On the other hand, gas chromatography is limited to volatile and semi-volatile compounds that can be vaporized without decomposition. It is less suitable for analyzing non-volatile compounds, high molecular weight compounds, and thermally labile compounds. Additionally, the technique requires reference standards for compound identification, and some compounds may have similar retention times, making definitive identification challenging. The application of gas chromatography is not that much limited because pharmaceutical industries, environmental analysis, forensics, food and beverage analysis, petrochemicals and many more industries use gas chromatography (Jackie & Shimadzu, 2020). It is frequently used to analyze organic substances that are volatile and semi-volatile. For all the following reasons, reverse phase HPLC was used for most of the time rather than normal phase HPLC or Gas Chromatography.

Then, second most recommended technique is Spectroscopy. This technique is also very important. It does not give accuracy like chromatography but it requires very short time. Spectroscopic methods are used to investigate the molecular interaction. This technique is

widely used in pharmaceuticals for drug identification, to know the purity level, acknowledge drug crystalline structure etc. It gives both qualitative and quantitative analysis data. Also direct, non-invasive and in-situ analysis can be done with this method. It is less expensive and requires short time to show the result. Very small amount of sample is needed to complete the assay and at the end of the reaction, the samples can be recovered. This method is very convenient with all the phases like solid, liquid and gas.

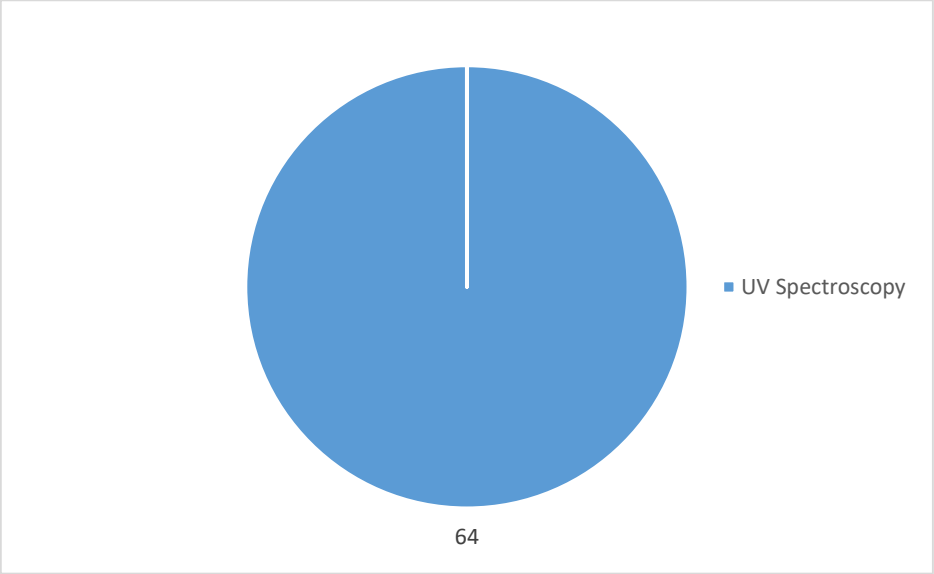

Figure 4: Spectroscopic Technique

From Figure 2, total 29% of the formulations studied, were recommended for spectroscopy and to be assigned by UV-vis spectroscopy. It is basically used to analyze the chemical properties of a substance. It is used to identify unknown substances, to calculate concentrations and to provide information on the physical and electrical compositions of both organic and inorganic substances. UV spectroscopy is a very quick process compared to other spectroscopic method. From the specific monograph studied, other spectroscopic techniques were not found to be recommended.

After spectroscopy, Titration technique has been recommended for several times which is almost 16% of the total. There are some reasons for which titration method is used for several times. The reasons can be- titration is an established analytical technique. Basically, it analyses purity and content. It is very fast, highly accurate and precise technique. It is relatively cheap and does neither require any specialized apparatus nor any specialized chemical knowledge. It offers a good performance ratio than any sophisticated technique. It can be used by low-skilled or low-trained operators. Titration with indicator is easier and quicker to perform. It is more convenient to use than other titrimetric technique.

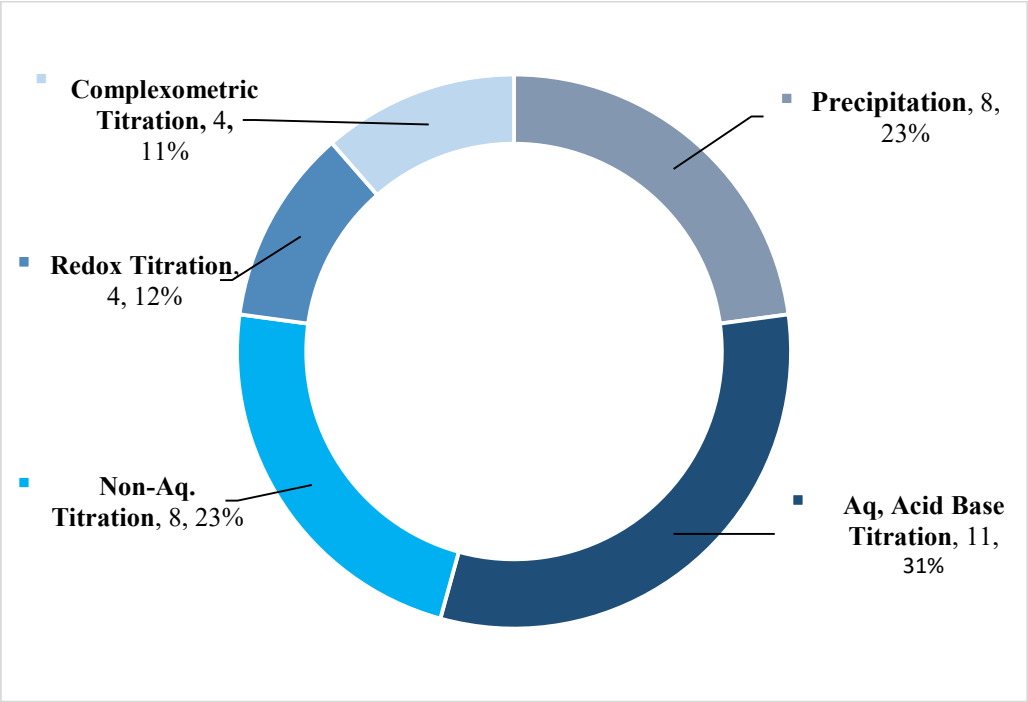

Figure 5: Titration Techniques

Among all types of titrations, Figure 5 sums up with 31% recommendation for aqueous acid-base titration. Then, non-aqueous titration and precipitation titration were recommended equally 23%. Additionally, redox titration and complexometric titration were also recommended 12% and 11% respectively. So, according to the observation, aqueous acid-base titration appears to be more useful than other titration techniques.

It is important to note that acid-base titration doesn't require specialized or expensive chemicals is its primary advantage. It just needs common chemicals like NaOH, KOH, HCl, acetic acid, ammonia etc. It is not expensive like others. The results are very accurate and precise. It is not time consuming and does not require any high expertise. It has a simple operating procedure and is used for quantitative analysis.

Major advantage of non-aqueous titration is that very weak acid and base can be analyzed easily. This method is also simple and accurate. Organic acid and bases that are insoluble in water, are soluble in non-aqueous solvent. The main benefit of precipitation titration, on the other hand, is that it is a widely used technique for determining the salt content of food, beverages and water as well as the presence of halide ions and specific metal ions in a solution. It can deliver an analysis' quick and accurate results.

Redox titration reacts with metal as it is an oxidizing agent. It simultaneously changes the concentration or making any decomposition. The speed of reaction is slow. It could generate chemical waste that has to be disposed of. It's not really accurate. This approach leaves room for human mistake.

Polarimetry technique has been recommended for the very least amount of time. It is very sensitive to scattering and motion. It has very low selectivity and causes noise. This method is time consuming and systematically energy-dependent. It also requires laser safety regulations.

Chapter 4

Conclusion

In the study, it has been focused on different types of analytical techniques like- spectroscopy, chromatography, titration on different types of monographs from British Pharmacopoeia, Vol. III. Also, it has been described about the assay sub-types of the monographs. It has been focused on why each assay is better and why so. There are total 1247 recommended formulation present in the BP. The study was done on a little fraction of portion which is only 220 drugs. This is the limitation that the database doesn't give a proper exact information. If the study was done on the whole database, it might give a better picture of a study. In the future prospect, the total database can be used that might give another prospect and much more information. The study can be used in educational purpose, for research and development purposes.

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