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Review on Development and Validation of Different Herbal Formulation by Using Various Chromatographic Methods

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ABSTRACT

The main aim of the review is to highlight different development and validation methods of different herbal formulation by various chromatographic method. Several phytoconstituent plant extracts or enhanced fractions rather than single phytoconstituent-based formulations are the major focus of the herbal formulation idea. As a result, methods for biological screening can be used to assess herbal medicines and formulations on a regular basis. Herbal medicines are available in a variety of forms depending on their intended function. Globally, Large-scale manufacture of these items is a result of the rise in demand for herbal medicines. The different chromatographic techniques are employed in the herbal formulation. The primarily employed technique is High Performance Liquid Chromatography.

KEYWORDS: High Performance Liquid Chromatograpy, Herbal Formulation, Curcumin and Gallic acid, Eugenol, Ellagic acid.

I. INTRODUCTION

It is frequently one of the initial steps in determining and comprehending the chemical composition of a target molecule or substance. Identifying possible contaminants or degradants in a formulation can also be done[1]. If time, money, and efficiency are issues, the idea of phase-appropriate method development is crucial. The objective and purpose should be consistent with the stage of drug development. Early drug development techniques might concentrate on Active Pharmaceutical Ingredient behavior. They ought to be capable of supporting pre-clinical safety assessments, pre-formulation investigations, and stability tests on prototype products. The analytical methodologies are improved and broadened as drug research advances, depending on greater understanding of the Active Pharmaceutical Ingredient and therapeutic product[2]. The techniques must be reliable and simple while also adhering to the necessary Regulatory Methods development, in the process of creating an analytical technique is, in accordance with Food and Drug Administration advice, "to test the drug substance or drug product against established acceptance criteria for a defined characteristic of that characteristic." Furthermore, even when developing a technique for non-regulatory objectives [3]. It can be important to have the intention of validating it later on. This can save a lot of time and money on future validation tasks. Specificity, linearity, limits of detection and quantitation, range, accuracy, and precision are all factors that the Food and Drug Administration advises including in a method development study [4]. According to Vincent Thibon, technical development lead, "method development consists of three main stages: feasibility where you determine whether the method will work with your sample; development where you optimize the method; and validation where the optimized method is validated to the relevant regulatory requirements." For herbal products, novel processes and reactions, novel compounds, active components, residues, impurity profiling, and component of interest in various matrices, methods development is necessary. Techniques, methods, processes, and protocols are all part of the method development process. Technique selection is the first step in method development, and effective method development necessitates professional knowledge and substantial practical experience[1]. Method development frequently leads to method validation. Numerous approaches can be used in method development. On the other end, it can entail modifying an existing technique by making tinier adjustments to make it work for a different application. It takes a lot of work, and there is some early uncertainty about the method's viability[3]. It entails working on a number of concepts concurrently before selecting one. The following are some of the steps in method development and validation. Throughout the pharmaceutical development process, dependable and reproducible

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analytical procedures are crucial. These methods must be able to gauge the finished drug's potency, purity, and stability. Nevertheless, a range of analytical approaches are useful to method development operations. Always use a well-developed approach for validation[2]. Any analytical approach that is used to estimate the drug requires the development of an appropriate methodology.

In an effort to raise the caliber of pharmaceutical products, two Food and Drug Administration named Ted Byers and Bud Loftus originally presented the idea of validation in the middle of the "Evaluating validation actions or proving effectiveness" is what the word "validation" signifies. Process validation is the process of gathering and analyzing data from the product design stage and throughout the process to demonstrate, by scientific proof, that the process can reliably produce high quality products[4]. It was put forth as a direct reaction to the significant number of issues with parental sterility products. The initial validation efforts were concentrated on the procedures used to create these drugs, but they quickly spread to all phases of pharmaceutical production. A new or improved method's integrity and quality must be ensured, including its transferability[1]. These factors include precision, accuracy, detection limit, limit of determination, selectivity, and linearity field. The Latin word Validus , which means strong, is the root of the word validation, which denotes that something has been established to be truthful, practical, and of a high standard .The process of demonstrating that a certain developed analytical method is suitable for its intended use is known as method validation .An essential prerequisite for the application of an analytical procedure is validation. Pharmaceutical Process Validation is one of the most significant and widely accepted requirements[3]. The quality regulation makes reference to the need for process validation. A quality system aims to consistently create goods that are suitable for the application for which they are designed. In order to ensure that these principles and the objective are achieved, process validation is a crucial component. In the quality assurance unit, validation is carried out using a formal, approved, and signed procedures validation protocol[2]. Validation is a technique that can be used in many disciplines, including sales, economics, psychology, chemistry, and biology. In fact, the validation idea is applicable in the majority of fields. Since validation operations involve checking manufacturing materials, operating procedures, training participants, and monitoring the system throughout production, the goal of validation is to test the quality of the system at each stage rather than just at the end[4]. Engineering procedures utilized in the delivery of major pieces of equipment that would be constructed, tested, delivered, and accepted in accordance with a contract were the foundation from which the concept of validation for equipment and processes was first formed. After a number of significant issues showed the possible hazards in product design, validation began to be used in other sectors of industry. Therac-25 is the most noteworthy occurrence. Here, the testing and design of the software for a sizable radiation apparatus were unsatisfactory. In usage, a number of interrelated issues caused some devices to deliver radiation dosages that were thousands of times higher than planned, which caused three patients to pass away and numerous more to sustain severe injuries. The anticipated outcome was reliably and consistently produced by the validated technique. It also emphasizes the analysis of the finished product and the product's conformity. It is a huge development for the pharmaceutical sector[1]. The consistency, accuracy, and dependability of the test sample's results are the goals of the analytical method's validation. Any approach can reveal issues, restrictions, and influence from outside sources the while doing the test. Therefore, these issues ought to be fixed. It is essential to attaining these objectives.

CHROMATOGRAPHIC METHODS

Greek words chroma, which means "color," and graphein, which means "to write," are the roots of the word "chromatography". It is a biophysical technique that is frequently used to separate various components in a mixture of liquids[5]. Due to their distinct capacities for adsorption in the stationary and mobile phases, components of a mixture can be separated by chromatography. This technique is regularly applied in chemical laboratories, pharmaceutical companies, and business. Even though there are various chromatographic processes, every chromatographic system has two components: a stationary phase that, in every case, consists of a solid surface or a liquid layer adsorbed on a solid surface, and a mobile phase that can either be liquid or gaseous. Sorting chromatographic techniques Chromatography is the name given to a certain class of highly efficient separation methods [6]. The name "chromatography" comes from Tswett, who first described the technique and used it to separate

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colored compounds. The restriction to colored compounds was never actually achieved, and today the majority of chromatographic separations are carried out on mixtures of colorless materials, including gases. Chromatography, like fractional distillation, depends on the relative movement of two phases, but in chromatography one of them is fixed and is referred to as the stationary phase, while the other is referred to as the mobile phase [7]. The goal of using chromatography, which is also used for quantitative analysis, is to achieve an acceptable separation in a reasonable amount of time. To that goal, numerous chromatographic techniques have been created. Among these are affinity chromatography, thin layer chromatography, column chromatography, paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, and high-pressure liquid chromatography. It is employed in a variety of academic fields, including chemistry, biology, and medicine. The chromatography process can be used to separate pigments, dyes, amino acids, vitamins, polymers.

The Analyte is mixed in a liquid or gaseous mobile phase, which is pumped through a stationary phase, in the separation technique known as chromatography. Usually, there are two phases: a Hydrophilic phase and a Lipophilic phase. These two phases interact with the analytes components in various ways [5]. They interact with the stationary phase for a longer or shorter period of time depending on their polarity, which causes them to be more or less retarded. The several components that are present in the sample are divided as a result. Each element of the sample elutes from the stationary phase at a specific time known as the retention time. By capturing and mapping the signal of the constituents as they pass the detector, a chromatogram is produced [6]. Various type of chromatography are follow, Anion chromatography, Cation Chromatography, Column chromatography, Flash chromatography, Gas chromatography Gel filtration chromatography/ Gel permeation chromatography/ Size exclusion chromatography Molecular sieve chromatography, High-performance liquid chromatography Hydrophobic interaction chromatography, Ion exchange chromatography, Liquid Chromatography, Paper chromatography, Reverse-Phase chromatography[7].

II. HERBAL FORMULATION

Herbal formulations are dosage forms that contain one or more herbs or processed herbs in precise amounts to provide specific nutritional or cosmetic benefits. They are used to treat illnesses in humans or animals, as well as to change their structure or physiology. Formulations made from herbs include an active ingredient as well as one or more herbal preparations as well as other herbal ingredients [8]. Herbal compounds are transformed into herbal formulations through processes like comminution or powdering, distillation, expression, fractionation, purification, concentration, or fermentation. Algae, fungi, and lichen were used in their natural, unprocessed state in the production of herbal preparations. These substances were normally dried but occasionally fresh. Herbal remedies are primarily used for chronic, as opposed to life-threatening, diseases and for health promotion. But when modern medicine falls short in treating an illness, as it does with advanced cancer and newly developing infectious diseases, more people turn to traditional therapies [9]. Elements influencing the herbal preparation of

Long-term variations in product quality and stability are caused by environmental factors such as rainfall, temperature, altitude, soil, and storage conditions, as well as different harvesting techniques, timing, and methods of collection, manufacturing processes such as selecting, drying, purifying, and extracting, and genetic variation Bitterness value, hemolytic activity, astringency, Sterling index, and foaming index are the pharmacological parameters for herbal preparations. Toxicological: Metals, arsenic, and pesticide residue. Pathogens, Aflatoxins, radioactive contamination, and total viable count are examples of microbial contamination. Replacement of fake medications and adulteration. Tinctures, extracts, essential oils, expressed juices, and processed exudates are a few different herbal preparations. benefits of herbal remedies - Since herbal medicine is a natural substance, our bodies can benefit from it[8]. Patients can gradually lessen or even get rid of the daily side effects associated with prescription medication by switching to a natural alternative herbal formulation goods in herbal products, Indian herbs used in ayurveda, Churna and Goli are ayurvedic, Tablets of glutathione, Juice from herbs, botanical extracts, Powdered herbs, Herbal & Ayurvedic Powder. In India, herbal formulations for Nootropies, Antidiabetics, Hepatoprotective drugs, and cholesterol lowering medicines have attained broad

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acceptance as medicinal treatments. Nowadays, herbal medications are more popular, especially for the treatment of Type 2 diabetes [9]. Although there are several ways to lessen diabetes' negative effects and its secondary problems, Herbal remedies are popular since they are less expensive and have fewer adverse effects. This review examines the pharmacological studies of several polyherbal formulations as well as their potential for treating diabetes. "Polyherbal Formulations" are pharmacological preparations that combine multiple herbs in order to boost therapeutic potency and lessen individual herb toxicity [8]. If the markers have been quantitatively determined in the herbal substance or herbal preparations .they are used to compute the amount of herbal substance or herbal preparation in the herbal formulation.

2.1 CURCUMIN AND GALLIC ACID

For the simultaneous measurement of curcumin and gallic acid from multi-herbal dosage forms, a straightforward, swift, exact, and affordable reverse phase High Performance Liquid Chromatography approach has been established. The procedure was done using a monolithic C18 column, and the mobile phase was made up of methanol, 0.1 percent tri fluoro acetic acid. At 1.0 ml/min flow rate. At below 300nm detection was done. Gallic acid and Curcumin had retention times lesser than 4 minutes and 2 minutes, respectively [10]. In terms of accuracy, precision, linearity, limit of detection, and limit of quantitation, the devised method was verified. The calculation of these medications in dosage forms that contain multiple herbs is possible using the suggested procedure. By measuring loss, the formulation of capsules was standardized. Curcumin. Obtained from the rhizome of the Curcuma longa plant, a member of the family Zingiberaceae[11]. Chemically speaking, Curcumin is 1,7-bis(4-hydroxy3-methoxyphenyl)-1,6-heptadiene-3,5-dione and exhibits a number of biological properties including antibacterial, anti-protozoal, antiviral, hypoglycemic, anticoagulant, antioxidant, antitumor, anti-carcinogenic, coloring, and flavoring. Gallic acid, also known as 3,4,5-trihydroxybenzoic acid, is a phenolic acid that is derived from the fruit of Embelica officinalis, a member of the Euphorbiaceae family.

The pharmacological effects of Gallic acid include anti-inflammatory, antibacterial, antifungal, anti-diabetic, anti-cancer, antioxidant. and antiviral propertiess.

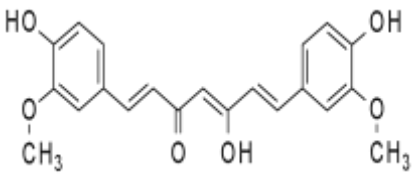

Fig 1: Curcumin

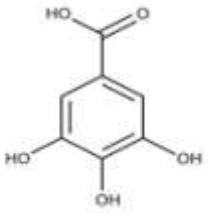

Fig 2 : Gallic Acid

Finar Chemicals Ltd. in Ahmedabad provided in the Chromatography grade methanol that was purchased. Finar Chemicals Ltd. in Ahmedabad provided the trifluoroacetic acid that was purchased. High Performance Liquid Chromatography grade for water was derived from a water purification system by Milli-QRO[12]. Gallic acid and curcumin reference standards are obtained from Gandhi Nagar's Aum Research Laboratories. Using chromatographic separation, technique for separating colors outfitted with an isocratic pump for the Chromatography system, Clarity 3.01 data station was equipped with an auto injector volume. Applied for the processing and collection of data. A solitary C18 10 cm long, 4.6 mm in diameter, and 5 m-sized particle column utilized to create the divide.

According to World Health Organization recommendations, the marketed formulation was standardized. We performed the parameters of moisture content, ash value, and extractive value.
Reverse Phase –High Performance Liquid Chromatography was used to estimate Curcumin and Gallic acid in dose forms under ideal chromatographic conditions [13]. The sample solutions is prepared and recorded.

2.2 Monoammonium Glycyrrhizinate And Sennoside B

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Fig 3: Senna leaf (Sennoside B)

Although it is urgent, standardizing Polyherbal medicine is a laborious undertaking.Phytochemical profiling is one of the many techniques used to standardize and control the quality of Polyherbal medication since it indicates the medicine's effectiveness and quality[14]. The goal of the investigation was to create and validate a straightforward, speedy, and precise Reverse Phase -High Performance Liquid Chromatography method for simultaneous. A polyherbal laxative tablet's monoammonium glycyrrhizinate and sennoside-B are evaluated. Plant biomarkers were successfully measured by the Chromatography on a C18 analytical column with a gradient mobile phase made up of phosphate buffer to aceto nitrile, and 254 nm as the detector wavelength [15]. The developed technique was verified. by determining factors such as precision, limit of detection, linearity, accuracy, and quantification.

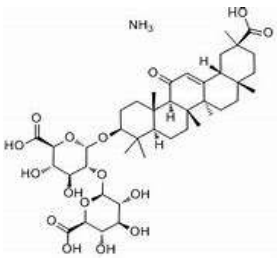
Fig 4: Monoammonium glycyrrhizinate

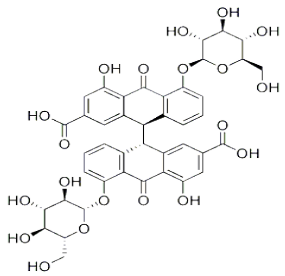
Fig 5: Sennoside B

For the simultaneous quantification of monoammonium glycyrrhizinate and sennoside-B in polyherbal formulations and plant materials, a new Chromatography method is being investigated. Previous research has revealed that there are various techniques for quantifying Sennoside-B and monoammonium glycyrrhizinate separately. But there is no that Chromatography technique.enables the concurrent estimation of these phytomarkers in combination in any dosage forms [16]. The primary goal of the research is to provide a quick, dependable, and economical technique for such estimate that needs little sample preparation Sennoside-B and Monoammonium Glycyrrhizinate can be determined using documented methods in the literature both alone and in combination with additional medications from Swarnapatri, Yashtimadhu, and Aragvadhation. Excellent linear correlation coefficients were visible in both phytomarkers' calibration curves [17]. Also using an equation, Limit of Detection and Limit Of Quantification were determined. Relative Standard Deviation values were determined to be less than 2.00% in precision studies employing intra-day and inter-day intervals. When the formulation was spiked with the appropriate phytomarkers, the approach was shown to be accurate, as evidenced by the recovery of Monoammonium Glycyrrhizinate and Sennoside-B from 98.96 to 101.39% and 99.17 to 100.67%, respectively.

2.3 EUGENOL

A brand-new, straightforward High Performance Thin Layer Chromatography method was created and approved for the quantitative determination of Eugenol in herbal oil used to relieve muscular and joint pain. We utilized silica gel 60F-254 (0.2 mm thickness) precoated aluminum Thin Layer Chromatography plates [18]. In a twin-trough glass chamber filled with mobile phase, the linear ascending development was conducted. Tolune: An Ethanol to acetate (9.3:0.7) ratio was then determined densitometrically using a Thin Layer Chromatography scanner set to reflectance/absorbance mode at 560 nm. Rf was discovered to be 0.58. For the development of analytical methods and quantitative analysis, markers were employed. Baidyanath Life Sciences Pvt. Ltd. bought them from the vendor Natural Remedies Pvt Ltd. Tolune, ethyl acetate, methanol, and pet ether were all of analytical quality [19]. Aluminum plate 60 F254 with a pre-coated silica

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gel coating for the stationary phase was purchased from Merck in Germany.

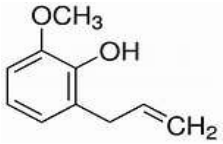

Fig 6: Eugenol

Eugenol in polyherbal muscle and joint pain relieving oil by High Performance Thin Layer Chromatography as the Chemically, Eugenol (4-Allyl-2-methoxyphenol)[Figure 6] is utilized as an analgesic, a biocide, and an antiseptic.The current study aims to develop a quick, effective, and repeatable method of analysis for literature survey8–16 clearly demonstrates that there is no proper High Performance Thin Layer Chromatography method available for the quantitative estimation of Eugenol in polyherbal muscle and joint pain relieving oil[20]. Instead, the majority of work has been reported on Reverse Phase –High Performance Layer Chromatography. The newly devised method, which may be used for routine quality monitoring of polyherbal oil that relieves joint and muscular pain, was found to be straightforward, precise, quick, and reproducible. The linearity and repeatability data of the medications employed in this method's testing revealed that there is no significant interference with the drug's estimation [21]. The procedure is relatively quick and easy to use; it doesn't call for tedious sample preparation or mobile phase setup. The suggested approach also demonstrated strong specificity and selectivity.

2.4. ELLAGIC ACID

For the purpose of estimating the presence of Ellagic acid in commercial herbal formulations, the High Performance Thin Layer Chromatography method known as Glucomap tablet was developed. This method employed a stationary phase of precoated Silica Gel F254 Plates and a mobile phase of Toluene, Ethyle acetate, Formic acid, and Methanol [22]. The wavelength of maximum Ellagic acid absorption is 280 nm, which was used to scan the developed chromatogram. By validating the devised High Performance Thin Layer Chromatography method in accordance with the International Council For Harmonisation criteria, its suitability for ellagic acid estimation was determined [23]. It was also investigated how much ellagic acid was included in the polyherbal formulation and crude medication Terminalia, arjuna, Ellagic acid in polyherbal formulations has been successfully determined using the suggested approach.

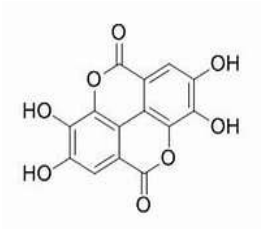

Fig 7: Ellagic Acid

The method's precision, accuracy, limit of detection, limit of quantification, robustness, ruggedness, and sample application specificity were all validated. The method's precision, accuracy, limit of detection, limit of quantification, robustness, ruggedness, and sample application specificity were all validated. For the purpose of determining the presence of ellagic acid in Glucomap tablets, the established High Performance Thin Layer Chromatography approach is exact, accurate, and reliable[24]. Statistics demonstrate that the ellagic acid analysis method is repeatable. The ellagic acid concentration of commercialized polyherbal products formulations used in laboratories are equivalent to formulations. This technique can be used to successfully analyze ellagic acid on a regular basis for the standardization and quality control of pharmaceutical goods that contain ellagic acid as an ingredient.

2.4.1 ELLAGIC ACID, BERBERINE AND FERULIC ACID

The goal of the current work was to create a novel, accurate, and straightforward High Performance Thin Layer Chromatography method for standardizing the three biomarkers berberine, ellagic acid, and ferulic acid in Amrtadi churna. Amrtadi churna, which contains dried Gokshur, Amla, and Guduchi plant pieces, is mostly used to treat hyperacidity [25]. Precoated silica gel at 60 F254 was used as the stationary phase in the method's development and validation, while toluene: ethyl acetate: formic acid: methanol was used as the mobile phase. At 319 nm, the detection and quantification were carried out, and the Rf values for Berberine, Ellagic acid, and Ferulic acid were respectively 0.35, 0.5, and 0.74[26]. The method's linearity, precision, specificity, accuracy, and robustness were all validated in accordance

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with International Council For Harmonisation criteria.

Fig 8 : Berberine

According to a review of the literature, the amounts of berberine, ellagic acid, and ferulic acid in plant extracts and commercial formulations were all evaluated separately. However, they weren't approximated simultaneously in any formulation. Therefore, the goal of the study was to create an High Performance Thin Layer Chromatography method that would be quick, accurate, and repeatable for assessing berberine, ellagic acid, and ferulic acid in Amrtadi churna[27]. The standards (Berberine, Ellagic acid, and Ferulic acid), as well as the raw medicine powders (Guduchi, Amla, and Gokshur), were purchased from Yucca Enterprises in Mumbai, India. As a stationary phase, silica gel 60F254 Thin Layer Chromatography plates (E Merck, Germany) were employed. All of the chemicals and reagents were purchased from Suvidhinath Laboratories in Vadodara, Gujarat, and were of analytical grade. For analysis, prepared Amrtadi Churna was employed. The developed approach underwent validation in accordance with Q2(R1)21 of the International Council for Harmonization criteria[25]. It is essential to be certain of the formulation's quality due to the variety of external factors and the polyherbal formulation's variable character. Therefore, the High Performance Thin Later Chromatography method was developed and validated for berberine, ellagic acid, and ferulic acid in order to guarantee the quality of the Amrtadi churna that was created.

2.5 ZOLPIDEM TARTRATE

A nonbenzodiazepine hypnotic drug, zolpidem tartrate (Zol-T), chemically known as N, N, 6-Trimethyl-2-ptolyl-imidazo(1,2-a)pyridine-3-acetamide L-(+)-tartrate (Fig.9), binds selectively to one benzodiazepine receptor subtype, benzodiazepine-1.[1-2] Without having any anticonvulsant or muscle-relaxing effects, Zol-T works as a sleep aid. When compared to High Performance Liquid Chromatography, high performance thin layer chromatography has various benefits [28]. Compared to High Performance Liquid Chromatography, it lowers the analysis's cost. High Performance Thin Layer Chromatography offers a very low mobile phase consumption per sample, which lowers the cost of acquisition and disposal. The High Performance Thin Layer Chromatography method was created and validated with consideration for the expense and applicability of analysis for estimation of zolpidem tartrate in bulk and its commercial formulation [29]. For the suggested analytical work, the Camag High Performance Thin Layer Chromatography system with Win CATS software was utilized. Silica Gel 60 F254 precoated Thin Layer Chromatography Plates were used to carry out planar chromatographic development. Linomat 5 applicator facilitated a sample application. Following sample application, 1010-dimensional twin trough chambers with 10 ml of solvent were used to put the plates to ascending development.

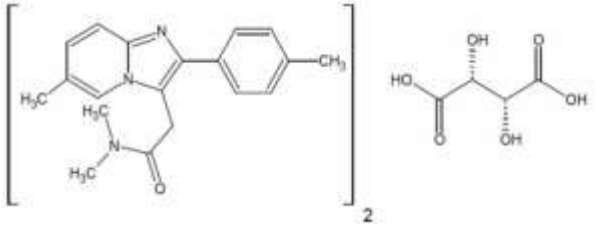

Fig 9: Zolpidem Tartrate

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According to "International Council For Harmonisation guidelines Q2(R1) for validation of analytical procedures: text and methodology," the created High Performance Thin Layer Chromatography method was validated[30]. Peak area against sample concentration was plotted, and a linear connection was discovered. According to the calibration graph, Zol-T demonstrated a linear response between 200 and 800 ng.

2.6 CURCUMIN, PIPERINE AND CHAMPHOR

To create a new, accurate, precise, and linear reverse phase high performance liquid chromatographic method for the simultaneous qualitative and quantitative estimation of curcumin, piperine, and camphor in an ayurvedic formulation, and to validate the method in accordance with the standards of the International Conference on Harmonization. chose an ayurvedic dental powder for the current study, which is utilized to maintain oral cleanliness[31]. It strengthens teeth and gums and is prescribed for several dental issues. Curcuma longa (zingiberaceae), Piper longum (piperaceae), Cinnamomum camphora (lauraceae), and other unprocessed medications are used in the chosen ayurvedic composition. Curcumin from Curcuma longa, piperine from Piper longum, and camphor from Camphora camphora were the three chemical producers chosen for quantification, one from each medicinal herb employed as raw materials. The physiological behavior A gift sample of High Performance Liquid Chromatography grade curcumin, piperine, and camphor was obtained from Yucca Enterprises in Mumbai, India[32]. An ayurvedic remedy called Patanjali Divya Dant Manjan was purchased at a nearby bazaar. Solvents of the HPLC grade were bought from Thomas Baker. This approach used the Reverse Phase-High Performance Liquid Chromatography Shimadzu model with "Lab Solution" software. Shim-pack High Performance Liquid Chromatography C18 analytical column was utilized for analyte separation of the corresponding plants is caused by these makers.

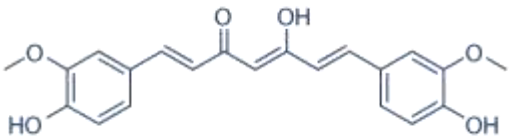

Fig 10: Curcumin

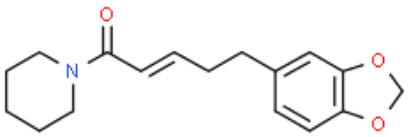

Fig 11: Piperine

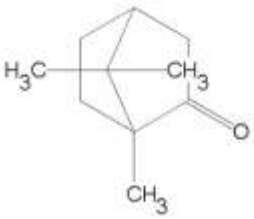

Fig 12: Camphor

The resolution factor between peaks, tailing factor, number of theoretical plates, runtime, and cost effectiveness were taken into consideration when developing a reverse phase High Performance Liquid Chromatogrpahy process[33]. The developed optimized approach led to the elution of curcumin, piperine, and camphor at 6.57, 7.32, and 8.57 minutes, respectively. The process of determining through laboratory tests if the method's performance characteristics satisfy the criteria for the intended analytical application is known as validation of the analytical method.

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According to International Council For Harmonisation requirements for the validation of analytical techniques, the designed High Performance Liquid Chromatography method was validated [31]. The method's linearity, accuracy, system precision, method precision, robustness, limit of detection, and limit of quantitation were all evaluated.

Fig 13: Camphor

Fig 14: Curcumin

Fig 15: Piperine

The findings show that a variety of markers are present in a particular ayurvedic dental powder, which may explain its therapeutic activity. The developed High Performance Liquid Chromatography approach will help standardize dental powder utilizing chemical markers that are biologically active.

2.7 SOFOSBUVIR AND VELPATASVIR

Sofosbuvir is chemically known as Isopropyl (2, 4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4- methyl-tetrahydrofuran-2-yl] methoxyphenoxyphosphoryl] amino] propionate (Figure 2.7.1). Velpatasvir is chemically known as Methyl 1-2- [(methoxycarbonyl)amino]-2-phenylacetyl}-4- (methoxymethyl)-2-pyrrolidinyl]-1H imidazol4- yl}-1, 11-dihydroisochromeno naphtho [1, 2-d]imidazol-2-yl)-5-methyl-1-pyrrolidinyl]-3- methyl-1-oxo-2 butanyl carbamate (Figure2.7.2) .Hepatitis C is treated with a combination of sofosbuvir and velpatasvir.. Sofosbuvir and Velpatasvir acting as an NS5B and NS5A inhibitor respectively[34]. In this study, a straightforward, quick, accurate, exact, specific, and sensitive Reverse Phase High Performance Liquid Chromatography technique for the simultaneous quantification of sofosbuvir and velpatasvir in bulk medication and pharmaceutical dosage form was developed and validated. Hypersil BDS column C18 was employed as the stationary phase for the chromatographic separation, while methanol:phosphate buffer was utilized as the mobile phase at a ratio of 75:25%V/V[35].

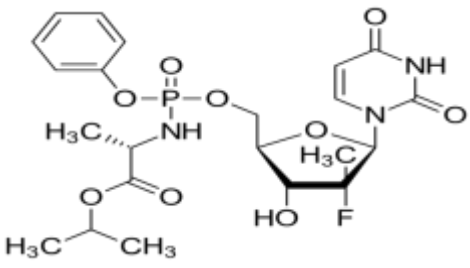

Fig 16: Sofosbuvir

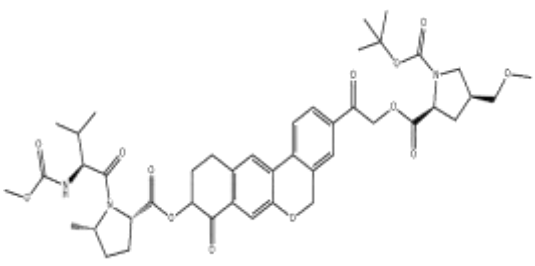

Fig 17: Velpatasvir

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Sofosbuvir and Velpatasvir were got as gift samples from Zydus health care center in Ahmadabad and hetero pharmaceuticals pvt, Ltd. in Hyderabad, India. They were pharmaceutically pure samples.The Samir Tech Chem supplied high performance liquid chromatography-grade methanol.. Pvt. Ltd[36]. From the experimental result the data for Accuracy was found to be between 98-102%, in the precision % RSD was found to be less then 2, all system suitability parameter was found within the acceptance criteria and Robustness was performed by deliberate changes in method parameters such as flow rate, mobile phase and pH but result obtained was within the acceptance criteria so it was concluded that, this newly developed method for the Sofosbuvir and Velpatasvir was found to be simple, precise, accurate, robust and high resolution and shorter retention time makes method more acceptable and cost effective[8]. In addition, it can be effectively applied for the routine analysis in research institution, quality control department and approved testing laboratories.

III. CONCLUSION

International Council for Harmonisation guidelines were followed in the method's validation. Five concentration levels, ranging from 300 to 900 g/ml for gallic acid and 10 to 30 g/ml for curcumin, were used to test the method's linearity. By using biologically active phytomarkers, the proposed and validated High Performance Liquid Chromatography approach will aid in the quality control of polyherbal laxative formulation. The newly devised method, which may be used for routine quality monitoring of polyherbal oil that relieves joint and muscular pain, was found to be straightforward, precise, quick, and reproducible. The method for analyzing ellagic acid is repeatable, according to statistical analysis. The devised High Performance Liquid Chromatography approach is accurate, exact, reproducible, and repeatable for the simultaneous measurement of curcumin, piperine, and camphor from ayurvedic dental powder.Shorter retention times make the newly developed approach for Sofosbuvir and Velpatasvir more palatable and economically advantageous. It was also found to be straightforward, exact, accurate, robust, and high resolution.

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