Method Development and Validation of Herbals: A Review

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ABSTRACT: The assurance of the safety, quality, and efficacy of medicinal plants and herbal products is turning into a vital issue as the demand for and commercial value of herbal medicines is rising dramatically. The development of a systematic strategy and thoughtful methodology for the standardisation of herbal raw materials and herbal formulations is urgently required. Standardization methods should take into consideration all aspects contributing to the quality of the herbal drugs. Herbal medicines are quite variable because they are made up of numerous ingredients. Hence, it is essential to create reliable chromatographic and spectroscopic fingerprints that represent the chemically unique and pharmacologically active components of herbal medicine. The data produced based on this chromatographic pattern may be used to identify genuine medications, weed out adulterants, and preserve the potency and consistency of the medication. This paper deals with the advanced chromatographic as well as spectroscopic techniques with the help of which qualitative and quantitative evaluation of Herbal Medicines and formulations can be carried out.

KEYWORDS: Standardization, Herbal Medicine, Chromatographic, Spectroscopic, Modification.

INTRODUCTION:

Traditional medical practises and medicinal plants are essential as a popular alternative to many pharmaceuticals. The search for new drugs has focused on medicinal plants since they are a rich source of bioactive compounds that are used to make drugs.[1] [Analytical chemistry is the study of the separation, measurement, and identification of chemical additives in synthetic and natural materials that are made up of one or more compounds or elements. The two main categories of analytical chemistry are qualitative evaluation and quantitative evaluation. Qualitative evaluation refers to the identification of the chemical additives present in the sample. Quantitative evaluation determines the amount of positive detail or compound present in the substance, i.e. the sample.[2] The advancement of a medication is a long procedure including drug invention, a research lab trial, preclinical testing, clinical testing, and regulatory registration. Require that the drug product is tested for its identification, potency, characteristics, quality, stability, and purity before it can be released for using in order to further improve the efficacy and protection of the medication after acceptance. Thus, pharmaceutical validation and process controls are vital in disregarding the issues that might be encountered. [3] The development and validation of analytical approaches play crucial roles in the research, development, and production of pharmaceuticals. The main aim of an analytical measure is to get consistent, realistic, and correct information. Validated analytical strategies play a significant role in achieving this goal. Results from methodology validation can be used to determine the quality, consistency, and dependability of analytical findings, which are essential components of any sane analytical procedure. Validation of analytical strategies is also needed by most rules and quality standards that impact laboratories. [4] It is crucial to properly validate conventional medical information using appropriate analytical methods of evaluation before integrating it into mainstream prescription-based medicine. However, it has been noted that while chemical entities from medicinal plants have supported the discovery of new drugs, formulations created over thousands of years offer priceless bioactive information that is still undiscovered. It is necessary to thoroughly characterise the bioactive metabolites of herbal drugs in order to produce a morden medicine from them. Reproducible results require the development, assessment and standardization of the chemical, biological and pharmacological methods. [5]

METHODS:

High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Ultra-Performance Liquid Chromatography (UPLC), Mass Spectroscopy (MS), Nuclear Magnetic Resonance Spectroscopy (NMR), Ultraviolet Visible Spectroscopy (UV),Infrared Spectroscopy (IR) are some methods developed and validated.

Development of Chromatographic method

Drug	Extract	Method	Acurracy (%)	Linearity (µg/mL-1)	LOD (µg/mL)	LOQ (µg/mL)	Correlation coefficients(r2)	Reference
Rubraxanthone	Stem bark of Garcinia mangostana Linn.	RP-HPLC	99.39-102.69	2.5-25	0.47	1.56	0.999	[6]
Da-Chai-Hu-Tang	Naringin	RP-HPLC	98.37	1.53-49.00	0.25	0.75	1.0000	[7]
	Hesperidin		98.70	2.70-43.20	0.20	0.48	0.9999	
	Neohesperidin		97.23	9.75-312.0	0.08	0.40	0.9999	
	Baicalin		101.20	6.25-200.0	0.07	0.35	0.9998	

	Wogonoside		98.13	3.25-120.0	0.18	0.50	0.9998	
	Baicalein		96.40	1.25-40.00	0.30	0.55	0.9994	
	Wogonin		98.08	3.12-50.00	0.30	0.74	0.9996	
	Emodin		99.75	4.00-64.00	0.25	0.87	0.9993	
	Chrysophanol		98.11	2.38-76.00	0.28	0.74	0.9992	
Vitexin	Passiflora foetida	HPTLC	97.83-99.33	100-700	6.51	15.12	0.9966	[8]
Cryptolepine	Cryptolepis sanguinolenta root	HPLC-UV	99.69-0.6641	10–200	6.74	20.42	0.9984	[9]
Ascaris lumbricoides	Gallic acid	HPLC-UV-MS	96.57-1.43	1-50	1.48	2.67	0.96	[10]
	Protocatechuic acid		90.13-0.63	1-50	1.26	2.34	0.97	
	hlorogenic acid		88.36-1.46	1-50	1.22	2.26	0.97	
	Caffeic acid		90.73-75.69	0.5-50	0.98	1.46	0.99	
	Rutin		87.83 - 7.56	0.5-50	0.89	1.74	0.96	
	Ferulic acid		96.89-2.64	1-50	1.12	1.98	0.99	
	Quercetin		100.11-0.78	1-50	1.13	2.40	0.98	
	Kaempferol		100.11-90.78	1-50	1.19	2.11	0.98	
Curcumin	Herabl extract	RP-HPLC	97.26-0.95	52-250	11.96	35.6	0.9989	[11]
Quercetin	Herbal extract	RP-HPLC	96.5-0.34	800-1600	32.94	99.76	0.9991	[11]
	Pushyanuga Churna	HPLC HPTLC	93.54-100.56	3.0-50.0	1.0	3.0	0.999	[12]
Berberin	Pushyanuga Churna	HPLC HPTLC	95.25-97.65	0.1-15.0	0.05	0.1	0.998	[12]
	Berberis aristata	HPLC-UV	95.98-98.02	569-1053	0.48	1.47	0.995	[13]
aristolochic acid	Aristolochia indica stem	HPTLC	100.02	91.02	3.3	10	0.998	[14]
Gallic acid	Polyherbal tablet extract	RP-HPLC	99.84	106.6-0.008	0.012	0.039	0.9977	[15]
Oleanolic acid	Polyherbal tablet extract	RP-HPLC	99.54	0.4-1.2	1.2116	3.6723	0.9979	[15]

CHROMATOGRAPHIC METHOD:

There are a variety of chromatography methods that have been used, such as thin-layer chromatography, liquid chromatography, and gas chromatography. Currently, most of the recent chromatography methods are embedded and combined with detectors, such as mass spectroscopy, Raman spectroscopy, and other detectors. Similarly, In plants, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs. Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as High- Performance Liquid Chromatography-Diode Array Detection onto a solid (HPLC-DAD), Gas Chromatography- Mass Spectroscopy (GC- are retained MS), Capillary Electrophoresis- Diode Array Detection (CE-DAD), bents, like High-Performance Liquid Chromatography-Mass Spectroscopy sins that can (HPLC-MS) and High-Performance Liquid Chromatography- used in the Nuclear Magnetic Resonance Spectroscopy (HPLC-NMR).[16]

Thin Layer Chromatography:

Thin Layer Chromatography (TLC) is one of the analysis methods performed through separation by chromatography. This method can be used for qualitative and quantitative analysis to identify samples of herbal medicine. This method is simple, fast, and the operating costs are inexpensive. Hence, it can be used in small laboratories to control adulterated drugs in herbal medicines. . The selectivity and sensitivity of TLC can be improved by selecting an appropriate detection for analysis. A recent study developed a thin layer chromatography method by combining TLC with Raman spectroscopy to increase the selectivity and sensitivity of the

analysis [17] Raman spectroscopy, especially surface-enhanced Raman spectroscopy (SERS), is a highly specific analytical technique that can be effectively used for qualitative analysis and chemical and physical structure elucidation [18,19]. Nonetheless, use of SERS is quite challenging when detecting analytes in the complex matrix, such as herbal products. Other than being coupled with SERS, the TLC method had also been developed with densitometric analysis, called TLC-densitometric. Densitometry is an instrumental analytical method based on the interaction of electromagnetic radiation with the analyte, which is a spot or stain on the TLC plate, for quantification purposes. The interaction of electromagnetic radiation with the stain on the TLC plate is determined to be the adsorption, transmission, or reflection of fluorine fluorescence, or the extinction of fluorine fluorescence from the original radiation. Other than being coupled with SERS, the TLC method had also been developed with densitometric analysis, called TLC-densitometric. Densitometry is an instrumental analytical method based on the interaction of electromagnetic radiation with the analyte, which is a spot or stain on the TLC plate, for quantification purposes. The interaction of electromagnetic radiation with the stain on the TLC plate is determined to be the adsorption, transmission, or reflection of fluorine fluorescence, or the extinction of fluorine fluorescence from the original radiation.[52]

In the phytochemical evaluation of herbal drugs, TLC is being ingredients and employed extensively for the following reasons: (1) it enables rapid to conventional analysis of herbal extracts with minimum sample clean-up offers some requirement, (2) it provides qualitative and semi quantitative information of the resolved compounds and (3) it enables the quantification of chemical constituents.[20]

Liquid Chromatography:

Another widely used chromatographic method is high-performance liquid chromatographic (HPLC), which can also be used for the separation of various components in the mixture. The separation principle of HPLC is based on the distribution of the analyte between eluent as a mobile phase and the packing material of the column as a stationary phase with high pressure through a column pump.[21] The use of HPLC can be combined with various detectors, such as ultraviolet detectors (UV) and mass spectroscopy (MS). HPLC-UV analysis techniques can provide sensitive and reproducible analytical results that have a fast analysis time, a low sample requirement, high accuracy, and precision to determine simultaneous compounds in the sample.[22]

Aside from being combined with a UV detector, HPLC can also be coupled with a tandem mass spectrometry (MS/MS). . The main principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds then separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance. Tandem mass spectrometry combines two mass analyzers in a single instrument to increase their abilities to analyze chemical samples. [23]

Liquid Chromatography- Mass Spectroscopy (LC-MS): In Pharmaceutical industry LC-MS has become method of choice in many stages of drug development. Recent advances includes electro spray, thermo spray, and ion spray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique.[24]

Liquid Chromatography- Nuclear Magnetic Resonance (LC- NMR): The combination of chromatographic separation technique with NMR spectroscopy is one of the most powerful and time saving method for the separation and structural elucidation of unknown compound and mixtures, especially for the structure elucidation of light and oxygen sensitive substances. The online LC-NMR technique allows the continuous registration of time changes as they processing in LC-NMR improves speed and sensitivity of detection. The recent introduction of pulsed field gradient technique in high resolution NMR as well as three-dimensional technique improves application in structure elucidation and molecular weight information. These new hyphenated techniques are useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process.[25]

Another liquid chromatography technique that can be used to detect adulteration of drugs is the ultra-high performance liquid chromatography (UPLC). UPLC operates at higher pressures (15,000 psi) and allows for lower particle sizes in columns, compared to HPLC that operates at lower pressures (max < 6000 psi). Both UPLC and HPLC have a similar accuracy and precision, however, UPLC has a better resolution, sensitivity, decreases in solvent consumption, and improves the quality of data. . UPLC is commonly combined with another detector, such as QTOF-MS or QTOF-MS/MS.[26,27] QTOF-MS is a hyphenated analytical technique that combines the benefits of two different mass analyzers, namely the time of flight and the quadrupole mass analyzer. This method combined the analyzers by utilizing the high compound fragmentation efficiency of quadrupole technology with the rapid analysis speed and high mass resolution capability of time-of-flight.[28]

Another research that used the UPLC-QTOF mass spectrometry method Instead of using a single mass spectrometry, it used tandem mass spectrometry (MS/MS) coupled with 2 mass analyzers by using a collision cell, for improving the specificity of the mass spectrometer.[27]

Gas Chromatography:

Besides LC and TLC, another method that can be used to detect the adulteration of herbal medicines is gas chromatography. It is a sensitive, reproducible, accurate, and has a lower cost compared to HPLC but is rarely used in comparison to TLC and LC because it requires additional pretreatment to achieve high thermal stability and has a volatile compound. The main characteristic that should be considered to analyze using gas chromatography is the volatility and thermal stability of the substances. Nitrogen (N2), hydrogen (H2), and helium (He) are three gases that are commonly used as carriers in gas chromatography (GC). Among those gases, the most commonly used carrier is helium. Helium is naturally found in gases and radioactive decay and is relatively rare in the atmosphere.[17] Hydrogen is used as an alternative gas carrier, in anticipation of a potential helium-shortage crisis, limited supply, and an expensive price in the future. Hydrogen offers some benefits for chromatography, including increased speed, lower temperature separations, longer column life, fewer environmental concerns, and greater availability.[29]

Gas chromatography is commonly coupled with mass spectrometry (MS) as a detector. The MS breaks each separate compound coming from the GC into ionized fragments, using a high-energy beam of electrons that are passed through the sample molecule to produce electrically charged particles or ions. A compound is analyzed by GC-MS, not only by comparing its retention time to a

standard (GC) but also by using its mass spectrum. The simplest mass detector in GC is the Ion Trap Detector (ITD). In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio frequency field; the trapped ions are then ejected from the storage area to an electron multiplier detector. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system.[30]

Gas Chromatography Fourier Transform Infrared spectrometry, Coupling capillary column gas chromatographs with Fourier Transform Infrared Spectrometer provides a potent means for separating and identifying the components of different mixtures.[43]

Development of Spectroscopic method:

Drug	**Extract**	**Wavelength selected (nm)**	**Accuracy (%)**	**Intraday**	**Interday**	**Correlation coefficients(r2)**	**Formulation**	**Reference**
				Precision %	**Precision %**			
Eugenia jamboloma	Seed	280nm	99.61%	0.397	_	0.996	Herbal capsule	[31]
Tagetes Erecta	Flower	369 nm	99.29%	0.015	0.02	0.999	_	[32]
Curcuma longa Linn.	Turmeric Standardised Extract	418 nm	98.41%	0.946	0.869	0.999	Polyherbal capsule	[33]
Apigenin	Salvia officinalis L.	268 nm	82.09%	0.0249	0.0757	0.0805	_	[34]
Eugenol	Toothpaste	204 nm	99.79%	0.631	0.633	0.999	Colgate Vedshakti	[35]
	Clove oil	282nm	98.87%	1.78	1.3	0.9914	_	[36]
Sitopala	plant	342.5nm	99.03%	0.978	_	0.9961	Sitopaladi churna	[37]
Berberine Hydrochloride	Berberis vulgaris	422 nm	92.27%	1.55	1.12	0.9996	Berbeshine tablet	[38]
Glycyrrhetinic acid	licorice	204 nm	99.03%	0.17	_	0.997	Pratishyayghna kwath	[39]
Embelin	Embelia ribes	289nm	98.96%	2.183	0.583	0.9991	Herbal formulation	[40]
Silicon	Eqisetum arvense L.	818nm	95.00%	2-12%	_	1.5	_	[41]
Azadirachta indica	Plant	271nm	99.84%	1.61	1.03	0.999	Herbal tablet	[42]

Curcuma longa Linn	Root	421nm	99.44%	0.39	1.39	0.9995	Herbal tablet	[42]

SPECTROSCOPIC METHOD:

A spectroscopic method is one of the widely used methods for detecting a component on a complex matrix, including adulterated drugs in herbal medicine. The spectroscopic methods that are commonly used include infrared spectroscopy, mass spectrometry (MS), and NMR spectrometry.[43]

Infrared Spectroscopy:

Infrared (IR) spectroscopy is the simplest, most rapid, and non-destructive analytical method without any previous sample pre-treatment.[44] IR spectroscopy is used to determine structures and functional groups of compounds and identify them based on the absorption by a molecule of a particular type of light, in the IR region of the electromagnetic spectrum. Fourier-transform infrared spectroscopy requires a mathematical process called Fourier transform to convert the raw data into the actual spectrum. The Michelson interferometer, which is the core of FTIR spectrometers, is used to split one beam of light into two, so that the paths of the two beams are different. Then the Michelson interferometer recombines the two beams and conducts them into the detector where the difference of the intensity of these two beams is measured as a function of the difference of the paths.[45,46]

Nuclear Magnetic Resonance (NMR) Spectroscopy:

NMR spectroscopy is an analytical technique used to determine the content and purity of a sample, as well as its molecular structure, by taking advantage of the magnetic properties of certain nuclei. The basic principle behind NMR is that some nuclei exist in specific nuclear spin states when exposed to an external magnetic field.[45]

Low-field (LF) NMR is an emerging technique based on the use of a new generation of compact NMR. It presents an opportunity to replace costlier or destructive methods while utilizing non-deuterated solvents.[47]

Mass Spectrometry (MS):

Mass spectrometry (MS) is an analytical technique that is used to measure the massto-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio.[48,49] Compared to other methods, MS can be used to detect a wider range of compounds.

Wooden-tip ESI-MS (WT-ESI-MS) is a technique that could be used for the direct analysis of raw samples. This technique makes use of readily available, economical, and disposable wooden toothpicks, which can be directly compatible with commercially available nano ESI ion sources, for sampling and ionization. The slim and hard wooden tips are very convenient for sampling, and the technique could be used for the analysis of samples of various forms.[50]

MS, was developed with ultrasonic extraction and nebulization in real-time, coupled with carbon fiber ionization (UEN/CFI-MS), to screen antidiabetic drugs, an antihypertensive drug, and hypolipidemic drug adulteration in herbal products. UEN/CFI is a pretreatment method used to ionize samples without adding auxiliary gas or a heating system. Compared with electrospray ionization (ESI), UEN/CFI has shown great compatibility with both polar and non-polar compounds. UEN and CFI were separated as two independent ionization sources. UEN was used as the ultrasonic extraction and nebulization device that acts to efficiently desorb the analytes from the sample.[51]

Uv Spectroscopy:

The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light or visible light by sample or chemical substance which results in the production of different spectra.[52] UV-Vis spectrophotometric methods assessing TPC in plant extracts are cheaper, faster, and thus more accessible methods than analytical chromatography techniques, such as high-performance liquid chromatography (HPLC).[53] Among different spectrophotometric techniques, UV-Vis spectroscopy appears to be suitable for the quantification of phenolic contents in the plant extract.[31] Phenolic compounds contain π-conjugated systems with hydroxyl-phenolic groups. They can strongly absorb UV light where π type molecular orbitals electronic transitions of phenolic groups provide the UV-vis spectrum.[54]

The instrument used was a SHIMADZU model 1800 (Japan) UV Visible spectrophotometer with a spectral width of 2 nm, a wavelength precision of 0.5 nm and a pair of 10 mm matched quartz cells to test the absorption of all the solutions.[32] Jasco double beam UV-Vis spectrophotometer (Model V-630) with 1.5 nm spectral bandwidth using 10 mm matched quartz cuvettes. Data acquisition was performed by using spectra manager software version 2.0. Secom am single beam UV-Vis spectrophotometer with 2 nm spectral band width using 10 mm matched quartz cuvettes.[33] for estimation process.

NEED FOR METHOD VALIDATION:

Method Validation is Necessary for the Following Reasons:

For assuring the quality of the products, for achieving the acceptance of the products by the international agencies, It is a mandatory requirement for accreditation as per ISO 17025 guidelines, A mandatory requirement for registration of any pharmaceutical product. Some of the organizations governing the quality standards are; United States of Food and Drug Administration (USFDA), Good Laboratory Practice (GLP) regulations, World Health Organization (WHO), The Pharmaceutical Inspection Cooperation Scheme's (PIC/S), The International Conference of Harmonization (ICH).[56,57] Validated methods are only acceptable for undertaking proficiency testing. Validation not only improves the processes but also confirms that the process is properly developed. [58,59]

The recent U.S. Food and Drug Administration (FDA) method validation guidance documents [60] as well as the United States pharmacopoeia (USP) both refer to ICH guidelines[61]

Validation is a continuous process, and it should comprise at least four steps for an analytical method validation; Planning and performing the tests, Statistical evaluation of the results, Report on the validation parameters, Application of all information gained during validation, Full validation processes and their explanations.[62]

Validation is a very important factor in controlling the reliability of the method that is determined by the validation results, where accuracy, sensitivity, applicability, specificity, the limit of detection, and the limit of quantification are reported. Validated

analytical methods play a key role in achieving the quality and safety of the final product, especially in the pharmaceutical industry.[63]

CONCLUSION: -

The methodology is developed by using the information obtained from the literature. Few times, there is a need to include extra instrumentation to reproduce, modify, validate or improve available methods for samples and analytes. For routine analysis in the laboratory, the use of instrument techniques, such as chromatography-based and spectroscopic-based with various detectors, or coupled with another detection method, is most likely. The instrumental technique provides excellent accuracy, precision, and sensitivity in the processes. The need of high-technology oriented applications has given rise to investigate and offer highly advanced detected hyphenated techniques which will serve as a rapid and unambiguous tool in the herbal research and will also benefit the entire pharmaceutical.

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