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Chromatographic and Spectral Fingerprinting Standardization of Traditional Medicines: An Overview as Modern Tools

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Abstract: Herbal drugs must be marketed as safe and therapeutically active formulation whose performance is consistent and predictable new and better medina plant agents are being produced at an accelerated rate. At the same time more exacting and sophistical analytical tools and methods are being used their evaluations and elites source identification. Based on the conception of phytoequivalence, the chromatographic and spectral fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines. Several novel chemometric methods for evaluating the fingerprints of herbal products. Chemical fingerprints obtained by chromatographic HPLC. HPTLC GC-MS-LC-MS, FT- IR techniques, are strongly recommended for the authentaion of traditional herbal medicines.

Key words: Finger-printing traditional medicines chemometrics

INTRODUCTION

Herbal medicines have being used by billions of people around the world for thousands of years. Unfortunately, the quantity and quality of the safety and efficacy data on herbal medicine are far from sufficient to meet the criteria needed to support their use worldwide. One of the reasons is lack of adequate or accepted research methodology for evaluating herbal medicine. The existence of many complex phytochemicals makes it difficult to identify those that provide main therapeutic effect. It is common thought that the therapeutic effect of herbal medicine can be attributed to the interactions of multiple phytochemicals. Thus, taking into account as many as possible components is commonly used to evaluate herbal medicine. Increasingly issue of quality and standardization in particular, as the key feature differentiating otherwise generic herbal products. Marketing campaigns have emphasized the use of modern scientific techniques to ensure that products contain specified amounts of

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marker compounds, asserting that this process of standardization guarantees identity, safety, efficacy and stability. For the vast majority of botanicals, however, it is impossible to determine potency measuring such marker compounds, since the active principles have neither been conclusively identified nor can they be accurately measured. In order to overcome this issue, some manufacturers have attempted to measure a herbal product as a whole. The evaluation of a product in its entirety, so-called fingerprinting can be accomplished by appropriate methods, which may include HPLC-UV(DAD), HPLC-ELSD, HPLC-MS, GC-MS, HPTLC-densitometry, FT-NIR, high-field NMR or a combination of these techniques, chromatographic and spectral fingerprints analysis of herbal samples approach using various chromatographic and spectral techniques has become the most popular and potent tools for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs in all over the globe. In recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and its products and therefore be used for authentication and identification of the herbal products. Based on the concept of phytoequivalence, the chromatographic fingerprints of herbal medicines could be utilized for addressing the problem of quality control of herbal medicines (Liang *et al*., 2004; Andola *et al*., 2010a). By definition, a chromatographic fingerprint of a herbal medicine is, in practice, a chromatographic pattern of pharmacologically active and or chemically characteristic constituents present in the extract. This chromatographic profile should be featured by the fundamental attributions of integrity and fuzziness or sameness and differences so as to chemically represent the herbal medicines investigated. This suggest that chromatographic fingerprint can successfully demonstrate both sameness and differences between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines all over the worlds considering multiple constituents present in the herbal medicines and its products. The recent trend of implementation of good and rigid policies of quality management like ISO 9000 certification, Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP), Total Quality Management (TQM) and Validated instruments and services etc. is to provide a comfortable and congenial living atmosphere for the mankind with a better quality of life. After using the synthetic medicines for some decades and came across many difficulties in curing some diseases success fully, a relook was given to Traditional medicines, their strength and use for the diseases incurable with the existing methods of medicines.

WHY NEED DEVELOPMENT OF CHROMATOGRAPHIC AND SPECTRAL FINGERPRINTS

Herbal medicines play very important role in health care of 80% population of the words especially developing countries. As per the traditional medicines programme of the World Health Organization (WHO) nearly 80% of the world population use phyto-products, phytoconstituents of wild plants which play very important role in the livelihood of the rural communities (Dubey *et al*., 2004). Herbal medicine is very poplar in different system of

Medicines like Indian system of medicine, Chinese system of medicine, Unani Naturopathy, Oestropathy and Homeopath. All over the world plants play an important role in health care of majority of people? In India alone, nearly two million traditional health practitioners use plants for treatment of various ailments (Venkatesh, 2002). In recent years, interest on plant based drugs has increased considerably. Annual growth rate between 5-15% for trade of plant based drugs and raw material is indicative of growing demand for herbal drugs (ICS-UNIDO, 2004). But the quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. It is well reported that variation of quality and quantity of Medicinal plants need to developed method for fingerprinting (Andola *et al*., 2010b; Negi *et al*., 2009, 2010). Herbal drugs, singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs (World Health Organization). Traditionally only a few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines. However, the therapeutic effects of herbal medicines are based on the complex interaction of numerous ingredients in combination, which are totally different from those of chemical drugs. Thus many kinds of chemical fingerprint analysis methods to control the quality of herbal drugs have gradually come into being, such as thin layer chromatography, gas chromatography, high performance liquid chromatography etc. chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products. The entire pattern of compounds can then be evaluated to determine not only the presence or absence of desired markers or active constituents but the complete set of ratios of all detectable analytes. The chemical fingerprints obtained by chromatographic and electrophoretic techniques, especially by hyphenated chromatographies, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of herbal medicines and therefore be used for authentication and identification of the herbal products.

CHROMATOGRAPHIC TECHNIQUES FOR FINGERPRINT ANALYSIS OF HERBAL MEDICINES

When herbal medicines are concerned, there are always hundreds of components and many of them are in too low amounts. On the other hand, there usually exists variability within the different and even the same herbal materials. Consequently, to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components is not a trivial task. The performance of a chromatographic fingerprint obtained is closely dependent on the chromatographic separation degrees and concentration distribution of all chemical components in the herbal medicine investigated. Fortunately, chromatography offers very powerful separation ability, such that the complex chemical components in herbal medicine extracts can be separated into many relatively simple sub-fractions. Furthermore, the recent approaches of applying hyphenated chromatography and spectroscopy such as High-Performance liquid chromatography- photo diode array detection (HPLC-PAD), Gas chromatography-mass spectroscopy (GC-MS), capillary electrophoresis-diode array detection (CE-DAD), Liquid chromatography and mass spectroscopy(LC-MS) and HPLC-NMR, could provide the additional spectral information,

which will be very helpful for the qualitative analysis and even for the on-line structural elucidation. With the help of the spectral information the hyphenated instruments show greatly improved performances in terms of the elimination of instrumental interferences, retention time shift correction, selectivity, chromatographic separation abilities and measurement precision. If hyphenated chromatography is further combined with chemometric approaches, clear pictures might be developed for chromatographic fingerprints obtained. A chemical fingerprint obtained by hyphenated chromatography, out of question, will become the primary tool for quality control of herbal medicines (Lazarowych and Pekos, 1998).

ANALYTICAL TOOLS USED FOR CHROMATOGRAPHIC AND SPECTRAL FINGERPRINT TRADITIONAL HERBAL MEDICINES

Thin Layer Chromatography and High Performance Liquid Chromatography

Thin Layer Chromatography (TLC) is frequently used for the analysis of herbal medicines since various pharmacopoeias such as American Herbal Pharmacopoeia (AHP), Chinese Drug Monographs and analysis; Pharmacopoeia of People's Republic of China etc. still use TLC to provide First characteristic fingerprints of herbs. Rather, TLC is used as an easier method of initial screening with a semi quantitative evaluation together with other chromatographic techniques. High performance thin layer chromatography as the advantages of many-fold possibilities of detection in analyzing herbal medicines. In addition, HPTLC analysis is rather simple and can be employed for multiple sample analysis. The advantages of using HTLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, HPTLC is a method of determining the quality and possible adulteration of herbal products. It summarized the progress in forced-flow-planer chromatography (FFPC) and demonstrated the importance of the different techniques like rotation planar chromatography (RPC), overpressured-layer chromatography (OPLC) and electroplanar chromatography (EPC).

Gas Chromatography

Gas chromatography GC is usually used for fingerprint analysis when pharmacologically active components in herbal medicines are volatile chemical compounds. The advantage of GC clearly lies in its high sensitivity of detection for almost all the volatile chemical compounds. This is especially true for the usual FID detection and GC-MS. Furthermore, the high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times.

High Performance Liquid Chromatography

HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. It is necessary to notice that the optimal separation condition for the HPLC involves many factors, such as the different compositions of the mobile phases, their pH adjustment, pump pressures etc. Chromatographic finger printing the High performance Liquid Chromatograph was used. In the conventional UV-Visible detector, monitoring the samples at one or two selected wave lengths has a limitation of not being able to present the qualitative and quantitative information about the molecules present in the sample which are

not absorbing other than selected wave lengths. The photodiode array detector (PDA) will be scanning each of the ingredients eluted from the column from 200-800 nm, covering the entire range of UV visible electro magnetic radiation. This over comes the disadvantage of single wavelength analysis Thus, a good experimental design for the optimal separation seems in general necessary (Sanyal *et al*., 2003; Thanawiroon and Linhardt, 2003; Lin and Chen, 2003). In order to obtain better separation; some new techniques have been recently developed in research field of liquid chromatography. These are Micellar Electrokinetic Capillary Chromatography (MECC) High-Speed Counter-current Chromatography (HSCCC), low- pressure size-exclusion chromatography (SEC), reversed-phase ion-pairing HPLC (RP-IPC-HPLC) and strong amion exchange HPLC (SAX-HPLC) (Rice and Linhardt, 1989). They will provide new opportunities for good separation for some specific extracts of some herbal medicines.

Hyphenated Techniques

Combining a chromatographic separation system on-line with a spectroscopic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for the identification and/or confirmation of the identity of target/ wanted and unknown chemical compounds. For most (trace-level) analytical problems in research field of traditional herbal medicines, the combination of column liquid chromatography or capillary gas chromatography with a UV-VIS or mass spectrophotometer become the preferred approach for the analysis of herbal medicines. Various hyphenated procedures used for the analysis of herbal drugs are HPLC-DAD, CE-DAD, GC-MS, LC-MS, HPLC-MS, HPLC-DAD-MS, HPTLC-MS and LC-DAD-MS. The data obtained from such hyphenated instruments are generated two types of data and spectrum, which could provide much more information about wanted data. Technique are more reliable authentic as compared to classic one way chromatography and spectroscopy. A total analysis device has been recently demonstrated in the case of on-line HPLC-UV (DAD)-FTIR-NMR-MS analyses.

DIFFICULTIES IN DEVELOPMENT OF CHROMATOGRAPHIC AND SPECTRAL FINGERPRINTS FOR TRADITIONAL HERBAL MEDICINES

When herbal drugs are considered for analysis, a large number of chemical components are involved and many of them are in low concentration. Chromatographic instruments and experimental conditions are difficult to reproduce during real analysis. Thus, the baseline and retention time shifts surely will be in existence from one chromatogram to another. Many other problems associated with chromatographic fingerprints such as the occurrence of abnormal chromatograms from outlying herbal samples or experiments inevitably will be encountered. As a result, in order to obtain reliable chromatographic fingerprints, several data treatments would be needed during fingerprint analysis.

TRADITIONAL MEDICINES AND CHROMATOGRAPHIC FINGERPRINTS

Due to low toxicity and known pharmacological activity, traditional medicine, containing mainly of herbal drugs, has been popularly and extensively used for many centuries. However, it is not easy to conduct quality control and assurance of herbal medicines because of high variability of chemical components involved. According to World Health Organization (WHO), quality and quantity of safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use worldwide and there is

still a lack of adequate or accepted research methodology for evaluating traditional medicine upto now; traditional medicine has not been officially recognized in most countries. Under this situation, the U.S.F.DA does not definitely emphasize the developments of very clear pictures of all chemical components from herbal medicines and thus, the fingerprinting approach has been recently recognized and accepted for quality assessment of traditional medicines. The concept of phytoequivalence was developed in Germany in order to ensure consistency of herbal products. According to this concept, a chemical profile, such as chromatographic fingerprint, for a herbal product should be constructed and compared with the profile of a clinically proven reference product. Chinese State Food and Drug Administration have said to regulate the compositions of liquid injections with herbal ingredients using stringent quality procedures such as chemical assay and standardization. Fingerprints of herbal medicinal liquid injections are compulsorily carried out for this purpose. In addition, among the various experimental techniques, chromatographic methods are highly recommended for finding out fingerprints of herbal products because of the high separation ability of chromatography.

CHEMO METRIC APPROACHES AND DATA PROCESSING FOR CHROMATOGRAPHIC FINGERPRINT OF TRADITIONAL MEDICINES

Chemo metrics is a statistical approach to the interpretation of patterns in multivariate data. When used to analyze instrument data, chemometrics often results in a faster and more precise Assessment of composition of a product or even physical or sensory properties. For example, composition (fat, fiber, moisture, carbohydrate) of dairy products or grain can be quickly measured using near infrared spectroscopy and chemometrics. Food properties (e.g., taste, smell, astringency) can also be monitored on a continuous basis. In all cases, the data patterns are used to develop a model with the goal of predicting quality parameters for future data. The two general applications of chemometrics technology to predict a property of interest (typically adherence to a performance standard); and to classify the sample into one of several categories (e.g., good versus bad, Type A versus Type B versus Type C etc.). Chemometrics software is designed to recognize patterns in virtually any type of multidimensional analytical data. Chemometrics can be used to speed methods development and make routine the use of statistical models for data analysis. Keeping in view of the complexity of the chromatographic fingerprint and the irreproducibility of chromatographic and spectral instruments and experimental conditions, several chemometric approaches such as variance analysis, peak alignment, correlation analysis and pattern recognition were employed to deal with the chromatographic fingerprint. Many mathematical algorithms are used for data processing in chemometric approaches. The basic principles for this approach are variation determination of common peaks/regions and similarity comparison with similarity index and linear correlation coefficient. Similarity index and linear correlation coefficient can be used to compare common pattern of the chromatographic fingerprints obtained. In general, the mean or median of the chromatographic fingerprints under study is taken as the target and both are considered to be reliable. To facilitate the data processing, software named Computer Aided Similarity Evaluation (CASE) has been developed. All programs of chemometric algorithms for CASE are coded in METLAB5.3 based on windows. Data loading, removing, cutting, smoothing, compressing, background and retention time shift correction, normalization, peak identification and spectral matching, variation determination of common peaks/regions, similarity comparison, overly of sample classification and other data processes associated with the chromatographic fingerprint can be investigated with this software (Gong *et al*., 2005).

CONCLUSIONS

Traditional medicines world over have played an important role in the maintenance of human health and are existing for many centuries with out any side effects as compared synthetic medicines. The increasing use of herbal medicines demands the need of a good method of standardization for better regulation. The major problem of quality assurance of herbal medicines has been solved to a great extent with the help of chromatographic and spectral fingerprint analysis. The variation determination of common peaks/regions and spectra in a set of chromatographic fingerprints could provide effective information about qualitative and quantitative information on the characteristic components of herbal medicines investigated. On the other hand, whether the real samples were identified as the herbs with the same quality grade could be determined successfully by way of comparing the chromatographic fingerprints with the similarity index cluster analysis and linear correlation coefficient analysis. Furthermore, pattern recognition can be used to discriminate different kinds of samples of traditional herbal medicines investigated. Thus chromatographic fingerprint analysis serves as a promising quality control tool for traditional medicines.

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