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FINGERPRINT ANALYSIS OF HERBAL MEDICINES USING HPLC: AN OVERVIEW

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ABSTRACT

Fingerprint analysis approach using HPLC has become the most 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. It 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 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. It has been accepted as a tool to establish the Chinese medicine quality standard system.

Keywords: Phytoequivalence, Quality Assurance, Qualitative Identification, Quality Control.

INTRODUCTION

The construction of chromatographic 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 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. 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 globally considering multiple constituents present in the herbal medicines [1-4].

Objective

Fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evalution of quality and ensuring the consistency and stability of herbal drugs.

Significance

Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. But the quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. Herbal drugs,

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singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy [5]. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs [6]. 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. chromate graphic 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. Herbal drugs, singularly and in combination, contain myriad of compounds in complex matrices in which no single active constituent is responsible for overall efficiency. This creates a challenge in establishing QC standards for raw materials and standardization of finished herbal drugs. To determine not only the presence or absence of desired constituents but also the complete set of ratios can be determined [6-13].

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. Thus, a good experimental design for the optimal separation seems in general necessary [14-16]. 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) [17], high-speed counter-current chromatography (HSCCC), low- pressure size-exclusion chromatography (SEC) [18], reversed-phase ion-pairing HPLC (RP-IPC-HPLC)19,20 and strong anion exchange HPLC (SAX-HPLC) [19-21]. They will provide new opportunities for good separation for some specific extracts of some herbal medicines.

Procedure

High-performance liquid chromatography (HPLC) used commonly for developing fingerprint analysis of various herbal products are various columns like Alltima C_{18} column (250 mm × 4.6 mm, 5 μm),Kromasil C(18) column (250 mm x 4.6 mm, 5 microm), reserved-phase column (5 microm, 250 mm x 4.6 mm), Agilent Zorbax Extend C(18) , Zorbax C-18. Linear gradient elution used were water-acetic acid, 0.1% phosphoric acid and acetonitrile as mobile phase components at a flow rate of 0.8 ml/min,1.0m/min and detector wavelength at 254 nm ,245 nm, 280 nm, 205 nm.

OTHER COMBINATIONS

- HPLC-NMR(High Performance Liquid Chromatography-Nuclear Magnetic Resonance)
- HPLC-MS(High Performance Liquid Chromatography-Mass Spectroscopy)
- HPLC-DAD(High Performance Liquid Chromatography diode array detection)
- GC-MS(Gas Chromatography-Mass Spectroscopy)
- HP-TLC(Thin Layer Chromatography) is frequently used for the analysis of herbal medicines.
- For clear pictures hyphenated chromatography can be combined with chemometric approaches.

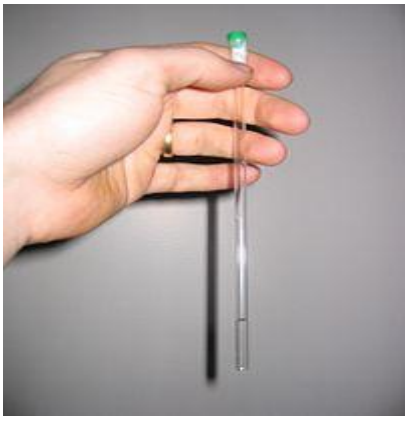
HPLC-Nuclear magnetic resonance spectroscopy

The most commonly known as NMR spectroscopy, is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The intra molecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule.

NMR spectra are highly unique, well-resolved, analytically tractable and often highly predictable for small molecules. Thus, in organic chemistry practice, NMR analysis is used to confirm the identity of a substance. Different functional groups are obviously distinguishable, and identical functional groups with differing neighboring substituents still give distinguishable signals. NMR has largely replaced traditional wet chemistry tests such

as color reagents for identification. A disadvantage is that a relatively large amount, 2–50 mg, of a purified substance is required, although it may be recovered. Preferably, the sample should be dissolved in a solvent, because NMR analysis of solids requires a dedicated MAS machine and may not give equally well-resolved spectra. The timescale of NMR is relatively long, and thus it is not suitable for observing fast phenomena, producing only an averaged spectrum [22-25].

Figure 1. The NMR sample is prepared in a thin-walled glass tube - an NMR tube.



When placed in a magnetic field, NMR active nuclei (such as ^{1}H or ^{13}C) absorb electromagnetic radiation at a frequency characteristic of the isotope. The resonant frequency, energy of the absorption, and the intensity of the signal are proportional to the strength of the magnetic field. For example, in a 21 Tesla magnetic field, protons resonate at 900 MHz. It is common to refer to a 21 T magnet as a 900 MHz magnet, although different nuclei resonate at a different frequency at this field strength in proportion to their nuclear magnetic moments.

In particular, the use of this on-line technology can remove the need for the isolation of the analyte in a pure form, thus improving both speed and efficiency.

HPLC- Mass Spectrometry

High performance liquid chromatography mass spectrometry is an extremely versatile instrumental technique whose roots lie in the application of more traditional liquid chromatography to theories and instrumentation that were originally developed for gas chromatography (GC). As the name suggest the instrumentation comprises a high performance liquid chromatograph (HPLC) attached, via a suitable interface, to a mass spectrometer (MS). The primary advantage HPLC/MS has over GC/MS is that it is capable of analysing a much wider range of components. Compounds that are thermally labile, exhibit high polarity or have a high molecular mass may all be analysed using HPLC/MS, even proteins may be routinely analysed. Solutions derived from samples of interest are injected onto an HPLC column that comprises a narrow stainless steel tube (usually 150 mm length and 2 mm internal diameter, or smaller) packed with fine, chemically modified silica particles. Compounds are separated on the basis of their relative interaction with the chemical coating of these particles (stationary phase) and the solvent eluting through the column (mobile phase). Components eluting from the chromatographic column are then introduced to the mass spectrometer via a specialised interface. The two most common interfaces used for HPLC/MS are the electrospray ionisation and the atmospheric pressure chemical ionisation interfaces [26].

HPLC-Diode Array Detector

Diode array adds a new dimension of analytical capability to liquid chromatography because it permits qualitative information to be obtained beyond simple identification by retention time.

There are two major advantages of diode array detection. In the first, it allows for the best wavelength(s) to be selected for actual analysis. This is particularly important when no information is available on molar absorptivities at different wavelengths.

The second major advantage is related to the problem of peak purity. Often, the peak shape in itself does not reveal that it actually corresponds to two (or even more) components. In such a case, absorbance rationing at several wavelengths is particularly helpful in deciding whether the peak represents a single compound or, is in fact, a composite peak.

In absorbance rationing, the absorbance is measured at two or more wavelengths and ratios are calculated for two selected wavelengths. Simultaneous measurement at several wavelengths allows one to calculate the absorbance ratio. Evaluation can be carried out in two ways:

In the first case, the ratios at chosen wavelength are continuously monitored during the analysis: if the compound under the peak is pure, the response will be a square wave function (rectangle),. If the response is not rectangle, the peak is not pure [27-29].

Gas Chromatography-Mass Spectrometry

GC-MS is an analytical method that identifies different substances within a test sample. The applications include drug detection, fire investigation and identification of unknown samples.Itcanalso be used in airport security to detect substances in luggage or on human beings.Aditionally, it can identify trace elements that were previously thought to have disintegrated beyond identification [30].

High Performance-Thin Layer Chromatography

This analytical technique provides more accurate and prescribed data.TLC studies are among the key identity test in most pharmacopoeial monographs.

HPTLC is robust, simplest, rapid and efficient tool in quantitative analysis of compounds. The separation can be further improved by repeated development of the plate, using a multiple development device. As a sequence HPTLC offers better resolution and lower limit of detection(LOD) [31].

EXAMPLE

Fingerprint Analysis of *Cirsium japonicum* DC Using HPLC

This herb is used as antihemorrhagic, anti-hypertension and anti-hepatitis agents and also used as a uretic in Chinese medicines.

In some areas people use Cirsium Setosum as Cirsium japonicam Dc because of same hemostatic activity.

The two herbs have similar appearance and similar compounds, they are totally different Medicinal materials, and have different pharmacodynamic actions.

The fingerprint spectrum can be a good tool to distinguish the two herbs and control the Quality of Cirsium japonicam DC.

Experimental

Materials and reagents: Methanol of HPLC grade, glacial acetic acid, deionosedwater, linarin.

Plant Materials

Roots of *Cirsium japonicum* DC and CirsiumSetosum . The collected plant materialsd were dried at room temperature in the absence of light in a well-ventilated room.

Instrumentation And Conditions

- Shimadzu LC-10AT HPLC
- Separation performed with SHIM-PACK VP-ODS column
- Water glacial acetic acid(100:1,v/v) and methanol used as mobile phase

Preparation of Standard Solutions

In a clean ,dry 50 ml volumetric flask, reference standard linarin was accurately weighed and dissolved in methanol to make reference be standard solution. It is stored at 4 Celsius.

Sample Preparation

Th**e** sample were gound into powder. 5.0g of the powder was reflux extracted with 70% ethanol at 70 for 2.5h. After filtering through a 0.45 micro meter membrane filter, the extract was transferred into a 100ml volumetric flask and adjusted to volume with 70%ethanol [32-37].

Method Validation

- The method validation mainly involved precision ,reproducibility and of the same ability.
- Precision was determined by replicate injection of the same sample solution for six times.
- Similarity of the 10 common chromatographic peaks was investigated with the software and it indicated 0.99 high precision.
- Reproducibility was determined by injection of six individual sample solutions extracted from the same sample in the same way.
- Stability was tested with one sample solution stored at room temperature for 0,3,6,9,12 and 24h,the similarity was calculated and exceeded 0.99 which indicated a good stability.

HPLC Fingerprints and Similarity Analysis

The samples had similar HPLC profiles

There were 10 characteristic peaks(from peak 1 to peak 10) which covered more then 90% of the total peak areas.

These results indicated that the samples shared almost the same correlation coefficients of similarities, showing the internal quality of these samples was excellent.

Relative Retention Time of the Common Peaks of *Cirsium japonicum*

Comparison of two similarity evalution methods:

- From the tables, it was found that all samples had good similarities, which were calculated with the software of Similarity Evalution System for Chromatographic Fingerprint of Traditional Chinese Medicine. This system could make qualitative identification of the samples.
- From below table, it was found that all samples were qualified with the third, fourth overall quantitative similarities, while the samples of no.4,7 and 15 were not qualified with the first and the second overall quantitative similarities. This method could simultaneously determine the contributions of big fingerprints to the system, and had the functions of qualitative and quantitative identification.

Overall quantitative and qualitative similarities of 15 batches of *Cirsium japonicum* DC.

Conclusion

An HPLC method was established for fingerprint analysis of Circiumjaponicum DC.

This method showed high precision and good repeatability and provided the basis for the quality control of Cirsiumjaponicm DC.

The fingerprints of *Cirsium japonicum* DC root, *Cirsium japonicum* DC carbon and Cirsiumsetosum was compared .It was found that the spectrum of *Cirsium japonicum* DC root, *Cirsium japonicum* DC carbon were somewhat different from that of *Cirsium japonicum* DC in peak retention time and peak relative area.

Cirsium japonicum DC root, *Cirsium japonicum* DC carbon had more small polar substances moreover,it was reported that *Cirsium japonicum* DC carbon had more

effective hemostasis, which indicated the hemostasis activity may be related to these small polar substances. Due to the same hemostasis activity, sometimes CirsiumSetosum is used as *Cirsium japonicum* DC. It was found that their HPLC spectrum had many differences and that the similarity was less then 0.5, which indicated that they were totally different plants [38].

APPICATIONS

- Mass spectrometry
- Community fingerprinting
- Brain fingerprinting
- Denaturation mapping
- Polymerase chain reaction
- Fingerprint powders

Mass spectrometry (**MS**)

It is an analytical technique that produces spectraof the masses of the atoms or molecules comprising a sample of material. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.In a typical MS procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons. This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection. The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern [39].

Community Fingerprinting

Itrefers to a set of molecular biology techniques that can be used to quickly profile the diversity of a microbial community. Rather than directly identifying or counting individual cells in an environmental sample, these techniques show how many variants of a gene are present. In general, it is assumed that each different gene variant represents a different type of microbe. Community fingerprinting is used by microbiologists studying a variety of microbial systems (e.g. marine, freshwater, soil, and human microbial communities) to measure biodiversity or track changes in community structure over time. The method analyzes environmental samples by assaying genomic DNA. This approach offers an alternative to microbial culturing, which is important because most microbes cannot be cultured in the laboratory. Community fingerprinting does not result in identification of individual microbe species; instead, it presents an overall picture of a microbial community.

Brain Fingerprinting

The technique uses the well-known fact that an electrical signal known as P300 is emitted from an individual's brain beginning approximately 300 milliseconds after it is confronted with a stimulus of special significance, e.g. a rare vs. a common stimulus or a stimulus the subject is asked to count. The application of this in brain fingerprinting is to detect the P300 as a response to stimuli related to the crime or other investigated situation, e.g., a murder weapon, victim's face, or knowledge of the internal workings of a terrorist cell Because it is based on EEG signals, the system does not require the subject to issue verbal responses to questions or stimuli. The person to be tested wears a special headband with electronic sensors that measure the EEG from several locations on the scalp. The subject views stimuli consisting of words, phrases, or pictures presented on a computer screen. Stimuli are of three types: 1) "irrelevant" stimuli that are irrelevant to the investigated situation and to the test subject, 2) "target" stimuli that are relevant to the investigated situation and are known to the subject, and 3) "probe" stimuli that are relevant to the investigated situation and that the subject denies knowing. Probes contain information that is known only to the perpetrator and investigators, and not to the general public or to an innocent suspect who was not at the scene of the crime. Before the test, the scientist identifies the targets to the subject, and makes sure that he/she knows these relevant stimuli. The scientist also makes sure that the subject does not know the probes for any reason unrelated to the crime, and that the subject denies knowing the probes. Since brain fingerprinting uses cognitive brain responses, brain fingerprinting does not depend on the emotions of the subject, nor is it affected by emotional responses. Brain fingerprinting is fundamentally different from the polygraph (lie-detector), which measures emotion-based physiological signals such as heart rate, sweating, and blood pressure. Also, unlike polygraph testing, it does not attempt to determine whether or not the subject is lying or telling the truth. Rather, it measures the subject's brain response to relevant words, phrases, or pictures to detect whether or not the relevant information is stored in the subject's .By comparing the responses to the different types of stimuli, the brain fingerprinting system mathematically computes a determination of "information present" (the subject knows the crime-relevant information contained in the probe stimuli) or "information absent" (the subject does not know the information) and a statistical confidence for

the determination. This determination is mathematically computed, and does not involve the subjective judgment of the scientist [40-46].

Figure 2. Use in criminal investigation

Figure 3.Dr. Lawrence Farwell conducts a Brain Fingerprinting test on Terry Harrington.

Figure 4. Dr. Lawrence Farwell conducts a Brain Fingerprinting test on serial killer JB Grinder.

Farwell's brain fingerprinting has been ruled admissible in court in the reversal of the murder conviction stated that the fundamental science involved in Dr. Farwell's brain fingerprinting P300 test was well established in the scientific community.

Limitations

Brain fingerprinting detects information-processing brain responses that reveal what information is stored in the subject's brain. It does not detect how that information got there. This fact has implications for how and when the technique can be applied.

In a case where a suspect claims not to have been at the crime scene and has no legitimate reason for knowing the details of the crime, and investigators have information that has not been released to the public, brain fingerprinting can determine objectively whether or not the subject possesses that information. In such a case, brain fingerprinting could provide useful evidence.

Another situation where brain fingerprinting is not applicable is one where the authorities have no information about what crime may have taken place. For example, an individual may disappear under circumstances where a specific suspect had a strong motive to murder the individual. Without any evidence, authorities do not know whether a murder took place, or the individual decided to take a trip and tell no one, or some other criminal or non-criminal event happened. If there is no known information on which a suspect could be tested, a brain fingerprinting test cannot be structured.

Another case where brain fingerprinting is not applicable would be one wherein a suspect and an alleged victim – say, of an alleged sexual assault – agree on the details of what was said and done, but disagree on the intent of the parties. Brain fingerprinting detects only information, and not intent. The fact that the suspect knows the uncontested facts of the circumstance does not tell us which party's version of the intent is correct [47-49].

In a case where the suspect knows everything that the investigators know because he has been exposed to all available information in a previous trial, there is no available information with which to construct probe stimuli, so a test cannot be conducted. Even in a case where the suspect knows many of the details about the crime, however, it is sometimes possible to discover salient information that the perpetrator must have encountered in the course of committing the crime, but the suspect claims not to know and would not know if he were innocent.

Denaturation Mapping

It is a form of optical mapping, first described in 1966. It is used to characterize DNA molecules without the need for amplification or sequencing. It is based on the differences between the melting temperatures of AT-rich and GC-rich regions [1]. Even though modern sequencing methods reduced the need for denaturation mapping, it is still being used for specific purposes, such as detection of large scale structural variants.

Methodology

When subjected to denaturing factors like increased heat or chemicals like formamide in low levels, DNA is partially denatured in a predictable pattern based on its nucleotide content in different regions. This allows unique fingerprints or 'barcodes' to be generated for molecules with different sequences not unlike restriction mapping. In the earliest forms of denaturation mapping, DNA was denatured by heating in presence of formaldehyde or glyoxal and visualized using electron microscopy. Dyes that selectively bind to double stranded DNA like ethidium bromide could be used to monitor the extend of denaturation. But it was not possible to observe locations of denaturation based on this information. And requirement of electron microscopy made this method more strenuous to perform. More recently microfluidics were used for denaturation mapping of single molecules. In this method a reservoir that contains individual stained DNA molecules is placed next to nanochannels. Under pressure the molecules are pushed towards the nanochannels, and stretch out. With the application of heat in the presence of formamide the molecules denature and

while in these channels, denaturation profile of these molecules can be observed by photolithography[50].

Historically, this method was used to compare and analyze properties of a single sequence or a group of sequences such as heterogeneity in the absence of sequencing. It was not uncommon to ribosomal DNA and compare the resulting maps of related organisms not unlike 16s rRNA identification method that is often used today [51-54] .But development of DNA sequencing techniques rendered this use of the method mostly obsolete.

Main advantage of denaturation mapping is that large-scale organization of the genome is left intact during the process. That means long-range structural variants can be detected easily.

Another recent application of denaturation of mapping is haplotype-phasing. Using a lab-on-a-chip approach, denaturation mapped single molecules can be rescued, amplified and analyzed by FISH and conventional sequencing. This approach yields a complete picture of chromosome at both macro and micro levels, allowing researchers to associate large structural variations with haplotypes [55].

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a biochemical technology in molecular biology used to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence [56-59].

- Initialization step: This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermo stable polymerases are used), which is held for 1–9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.

- Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
- Annealing step: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3–5 °C below the Tm of the primers used. Stable DNA–DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA formation.
- Extension/elongation step: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C,[12][13] and commonly a temperature of 72 °C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases per minute. Under optimum conditions, i.e., if there are no limitations due to limiting substrates or reagents, at each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.
- Final elongation: This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.
- Final hold: This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction [60-61].

Fingerprint Powders

Fingerprint powders are fine powders used in dusting for fingerprints by crime scene investigators and others in law enforcement. The process of dusting for fingerprints involves various methods intended to get the particles of the powder to adhere to residue left by friction ridge skin on the fingers, palms, or feet [62-65].

Physical development of fingerprints using powders is just one of a selection of methods used to develop fingerprints. Fingerprints often leave residues of oils in the shape of the friction ridges, but the friction ridge skin itself does not secrete oils, and so some fingerprints will only leave a residue of amino acids and other compounds which the powder does not adhere to well. For this reason, 'dusting' is used as part of an array of techniques to develop fingerprints, but is often used on larger areas in a crime scene which cannot be removed for analysis, or cannot be subject to more rigorous analysis for other reasons [66].

Table 1. Similarities of *Cirsium japonicum* DC from 15 different origins

Batch no	S1	S2	S3	S4	S5	S6	S7
1	1						
2	0.936	1					
3	0.997	0.932	1				

4	0.993	0.937	0.985	1			
5	0.892	0.851	0.916	0.878	1		
6	0.922	0.874	0.909	0.931	0.767	1	
7	0.985	0.947	0.98	0.985	0.879	0.917	1
8	0.996	0.946	0.996	0.992	0.914	0.916	0.987
9	0.963	0.918	0.975	0.955	0.978	0.858	0.954

Table 2. Relative retention time of the common peaks of *Cirsium japonicum*

Peak no.	1	2	3	4	5	6	7
1	0.199	0.199	0.199	0.199	0.1990	0.2	0.199
2	0.258	0.257	0.257	0.257	0.257	0.258	0.257
3	0.278	0.278	0.278	0.278	0.277	0.278	0.277
4	0.306	0.305	0.305	0.305	0.305	0.305	0.305
5	0.712	0.712	0.711	0.71	0.711	0.712	0.712
6	0.736	0.736	0.735	0.735	0.735	0.735	0.735
7	1	1	1	1	1	1	1
8	1.032	1.032	1.032	1.032	1.031	1.032	1.032

Table 3. Overall qualitative and overall quantitative similarities of 15th batch *Cirsium japonicum* DC

Bch no.	1	2	3	4	5	6	7	8
1	0.998	1.000	0.998	0.993	0.999	0.997	0.998	0.999
2	0.985	0.983	0.986	0.971	0/956	0.993	0.964	0.992
3	86.0	88.3	90.0	149.6	101.3	125.7	68,8	91.7
4	86.7	88.7	91.4	134.3	101.4	120.2	73.1	91.1
5	86.2	88.3	90.2	150.7	101.4	126.0	68.9	91.8
6	86.9	88.7	91.6	135.3	101.5	120.6	73.3	91.2
7	89.8	89.9	90.2	135.3	101.5	120.6	73.3	91.2
8	91.1	91.5	91.5	117.8	98.2	117.5	95.2	94.2
9	89.8	89.9	90.2	114.4	93.9	116.7	91.8	93.4
10	90.9	91.5	91.3	116.9	98.1	117.1	95.0	94.1

CONCLUSION

The problem of quality assurance herbal medicines can be resolved to a great extent with the help of chromatographic fingerprint analysis. Quality grade could be determined successfully by the way of determining chromatographic fingerprints with the similarity index and linear correlation analysis. The variation determination of common peaks in a set of chromatographic fingerprints could provide useful qualitative and quantitative information of componentsof herbal medicines investigated. Pattern recognition can be used to discriminate different kinds of samples 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 and linear correlation analysis. Thus, chromatographic fingerprint analusis serves as a promising QC tool for herbal medicines.

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