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Fingerprinting of complex mixtures with the use of high performance liquid chromatography, inductively coupled plasma atomic emission spectroscopy and chemometrics

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ABSTRACT

The molecular and metal profile fingerprints were obtained from a complex substance, *Atractylis chinensis* DC—a traditional Chinese medicine (TCM), with the use of the high performance liquid chromatography (HPLC) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) techniques. This substance was used in this work as an example of a complex biological material, which has found application as a TCM. Such TCM samples are traditionally processed by the Bran, Cut, Fried and Swill methods, and were collected from five provinces in China. The data matrices obtained from the two types of analysis produced two principal component biplots, which showed that the HPLC fingerprint data were discriminated on the basis of the methods for processing the raw TCM, while the metal analysis grouped according to the geographical origin. When the two data matrices were combined into a one two-way matrix, the resulting biplot showed a clear separation on the basis of the HPLC fingerprints. Importantly, within each different grouping the objects separated according to their geographical origin, and they ranked approximately in the same order in each group. This result suggested that by using such an approach, it is possible to derive improved characterisation of the complex TCM materials on the basis of the two kinds of analytical data.

In addition, two supervised pattern recognition methods, *K*-nearest neighbors (KNNs) method, and linear discriminant analysis (LDA), were successfully applied to the individual data matrices—thus, supporting the PCA approach.

1. Introduction

Fingerprint analysis has been accepted by the World Health Organization (WHO) as a strategy for the assessment of complex substances such as herbal and traditional medicines [1,2]. In general, different techniques have been applied to construct the fingerprints of traditional Chinese medicines (TCMs). These include high performance liquid chromatography (HPLC) [3–5], gas chromatography (GC) [6], thin layer chromatography (TLC) [7], high-speed counter-current chromatography (HSCCC) [8] and capillary electrophoresis (CE) [9]. However, commonly, only one technique has been applied at a time to establish a fingerprint, and this has restricted the information, which could be obtained from the sample.

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The World Health Organization estimates that 65–80% of the world's population uses traditional medicines as their primary form of healthcare [10], and TCMs form a large part of this market. This includes a wide range of herbal preparations worth billions of dollars annually [11,12]. Many TCMs are good examples of very complex substances, and as such they are ideal for research in the field of fingerprinting analysis.

Atractylis chinensis DC [13] is the rhizome of *Atractylodes chinensis* (DC) *Koidz*. It is well known for its low toxicity and multiple pharmacological activities. It has been used as a TCM to alleviate many diverse health problems [13], and is widely available in China in areas such as Jilin, Liaoning, Henan, Shandong, Shanxi, Inner Mongolia, Ningxia and Gansu provinces. However, there are many molecular components in the raw form of *A. chinensis* DC, which can attack the human nervous system [14]. Thus, *A. chinensis* DC must be carefully processed before it can be used as a medicine, and some typical treatments such as the Bran, Cut, Fried and Swill methods are described in Section 2.1.

Currently, fingerprint techniques, which are applied for quality control of TCMs, focus on product comparability, as reflected, for example, by the similarity of the chromatographic profiles of different batches of the TCMs or herbs. Very little work has been carried out to interpret or understand the differences in such profiles. The application of the combination of the two analytical techniques with the aid of chemometrics [15] for fingerprint analysis could be very useful. The approach provides complementary information from the TCM for quality assurance and authentication purposes.

In this paper, for the first time, we describe a study, in which the fingerprint of a complex substance/mixture such as a TCM, was established with the use of data derived from two techniques—HPLC results were combined with those collected from inductively coupled plasma atomic emission spectroscopy (ICP-AES) measurements. Thus, the data matrix was expanded to include both the molecular and the metal ion information found in the TCM. This potentially gave an information—enriched fingerprint provided all the data could be processed simultaneously. Therefore, the data matrix in this study, was submitted for data interpretation with the use of multivariate analysis and pattern recognition methods, such as principal component analysis (PCA), *K*-nearest neighbors (KNNs) method, and linear discriminant analysis (LDA).

2. Experimental

2.1. Plant materials

A. chinensis DC samples (total: 51) were purchased from different sources and chosen from different batches. The pharmaceutical providers were selected at random from five different provinces: Hebei, Jilin, Liaoning, Inner Mongolia and Shanxi during March 2005–March 2007 (Table 1). All the samples came from the northern parts of China, where *A. chinensis* (DC) *Koidz* plants may be found.

In general, the collected raw plant material has to be processed before it can be classed as a TCM. There are four common methods of processing:

Table 1 – Information on herbal TCM samples, *Atractylis chinensis* DC

Sample no.	Origin (province)	Batch no.	Processing method
1	Hebei	060309	Bran
2	Hebei	070101	Swill
3	Hebei	070311	Swill
4	Hebei	Unknown1	Swill
5	Hebei	Unknown2	Swill
6	Hebei	20070208	Fried
7	Jilin	0506002	Fried
8	Jilin	0603005	Swill
9	Jilin	0605001	Swill
10	Jilin	Unknown1	Swill
11	Jilin	Unknown2	Cut
12	Jilin	20061107	Bran
13	Liaoning	0601201	Swill
14	Liaoning	060306	Cut
15	Liaoning	060312	Fried
16	Liaoning	060901	Swill
17	Liaoning	061103	Fried
18	Liaoning	061202	Cut
19	Liaoning	061208	Bran
20	Liaoning	070310	Swill
21	Liaoning	20060413	Swill
22	Liaoning	20061114	Swill
23	Inner Mongolia	050302	Cut
24	Inner Mongolia	060508	Swill
25	Inner Mongolia	060702	Bran
26	Inner Mongolia	060802	Bran
27	Inner Mongolia	061003	Swill
28	Inner Mongolia	061015	Swill
29	Inner Mongolia	061209	Swill
30	Inner Mongolia	070207	Swill
31	Inner Mongolia	Unknown1	Swill
32	Inner Mongolia	20051208	Bran
33	Inner Mongolia	20060503	Bran
34	Inner Mongolia	20060901	Swill
35	Inner Mongolia	20060907	Swill
36	Inner Mongolia	20070101	Bran
37	Inner Mongolia	050602	Bran
38	Inner Mongolia	060108	Swill
39	Shanxi	060215	Bran
40	Shanxi	060313	Swill
41	Shanxi	060403	Swill
42	Shanxi	060506	Fried
43	Shanxi	061001	Swill
44	Shanxi	070311	Bran
45	Shanxi	Unknown1	Fried
46	Shanxi	20060306	Fried
47	Shanxi	20060408	Swill
48	Shanxi	20060724	Swill
49	Shanxi	20061003	Swill
50	Shanxi	20061108	Bran
51	Shanxi	20070102	Swill

i. The Bran (B) method: Husks or bran are heated in a pan till smoke is observed; then, the raw TCM is quickly added and fried until yellow. The mixture is then cooled and the TCM is separated from the husks by sieving.
ii. The Swill (S) method: The raw TCM is immersed in rice rinse water or swill. The TCM is filtered off, braised in a steam box and air-dried.
iii. The Cut (C) method: The raw TCM is cut into small pieces and air-dried.