Research on fingerprints of Chinese Material Medical to develop standard and research protocols evaluating their identity, safety, and reproducibility' 'Biopharma separation ' techniques'' - BL/03/C44 - BL/12/45

(Geographic) study area : China / Najing - Dalian

Data used:/

Context and objectives

Natural products are widely used in traditional medicine, and are nowadays also gaining interest in Western countries. Medicinal plants have a potential to be developed as high-value healthcare products. However, quality control is an important issue during development of such products to ensure the identity, safety and quality of the natural and synthetized products.
The project will have the following objectives:
- define fingerprint development methodologies in classic High-Pressure Liquid Chromatography (HPLC)
- data analysis of fingerprint data and definition of fingerprint analysis protocols
- isolation and characterization of individual herbal compounds
- define fingerprint development methodologies with miniaturized separation techniques
- define fingerprint development methodologies with two-dimensional HPLC
- compare HPLC fingerprints with metabolomic analysis by 1H NMR spectrometry and multivariate analysis techniques.

Methodology

- define fingerprint development methodologies in classic High-Pressure Liquid Chromatography (HPLC)
By the Flemish partner (VUB), a methodology to develop HPLC fingerprints for the quality control of herbal medicines was defined.

- data analysis of fingerprint data and definition of fingerprint analysis protocols
By one of the Walloon partners (Prof. Crommen), CE fingerprints were developed for 17 different samples of *Fructus Schisandrae chinensis* and a statistical comparison of these fingerprints is being performed.
By the Flemish partner (VUB), HPLC-DAD fingerprints were developed for 28 different samples of *Citri reticulatae pericarpium*. As data analysis, exploratory analysis using Principal Component Analysis is being performed. Secondly, it will be tried to develop a protocol to identify new samples as being *Citri reticulatae pericarpium* samples or not, based on their HPLC fingerprints.

- define fingerprint development methodologies with miniaturized separation techniques
By one of the Walloon partners (Prof. Crommen), an MEKC method was optimized to develop CE fingerprints of *Fructus Schisandrae chinensis*.
By the Flemish partner (VUB), it was tried to define a methodology to develop CEC fingerprints in one study, and in another study it is tried to define a methodology to develop p-CEC fingerprints. For the latter, both capillaries packed with silica particles as capillaries packed with monoliths are being tested.

- compare HPLC fingerprints with metabolomic analysis by 1H NMR spectrometry and multivariate analysis techniques
By one of the Walloon partners (Prof. Angenot), the samples of *Citri reticulatae pericarpium, Fructus schizandrae chinensis, Polygoni cuspidatae rhizome, Sinomenii acuti caulis, and Polygoni multiflori radix* were analyzed according to Pharmacopeial methods. Besides, NMR fingerprints will be developed.

Results

- define fingerprint development methodologies in classic High-Pressure Liquid Chromatography (HPLC)
A methodology to develop HPLC fingerprints was defined and an article concerning this methodology is in press in Acta Chromatographica.

- data analysis of fingerprint data and definition of fingerprint analysis protocols
At the moment, a statistical comparison of the developed CE fingerprints is being performed. This work is still ongoing.
At the moment, exploratory analysis is being performed on the developed HPLC-DAD fingerprints. The effects of different preprocessing techniques on the results of PCA are being evaluated, as well as where replicated samples from repeatability and time-different intermediate precision studies are located on the score plots. Secondly, it will also be tried to develop a protocol to identify new samples as being *Citri reticulatae pericarpium* samples or not, based on their HPLC fingerprints. For this purpose, three issues will be examined, i.e. (1) the identification of a sample from the HPLC fingerprint, (2) the choice of a suitable chromatographic column (is not allowed to be specified in Pharmacopoeias) and the effect of the column choice on the fingerprints, and (3) the robustness of the fingerprint. This work is still ongoing.

- define fingerprint development methodologies with miniaturized separation techniques
CE fingerprints were developed for 17 different samples of *Fructus Schisandrae chinensis* and the separation window of the MEKC system was determined using two appropriate markers.
It was tried to develop CEC of *Citri Reticulatae Pericarpium*. Capillaries packed with 5 µm ODS particles were used and the following experimental conditions were evaluated: the organic modifier, % MeOH, applied voltage, voltage gradient, buffer pH, and using a ternary mobile phase. It was found that the buffer pH and the % MeOH improved the resolutions in the fingerprints, but nevertheless, a gradient elution program is needed to obtain acceptable fingerprints.
Therefore, p-CEC fingerprints using capillaries packed with silica particles were evaluated. It is possible to develop p-CEC fingerprints, but the samples need to be more concentrated than in HPLC, and a set of 'good practices' (i.e. using extensive rinsing procedures, long column regeneration times and a strict monitoring of the column performance) is needed. To generate p-CEC fingerprints, the main drawbacks are the modest column robustness and the lengthy procedures. Hence, advances in the stationary phase technology and pCEC-equipment are necessary to utilize p-CEC to develop fingerprints. At the moment, p-CEC fingerprints using capillaries packed with monoliths are being evaluated.

- compare HPLC fingerprints with metabolomic analysis by 1H NMR spectrometry and multivariate analysis techniques
At the moment, the samples are still being analyzed according to Pharmacopeial methods, and NMR fingerprints are being developed. This work is still ongoing.

Products and services

Published

B. Dejaegher, G. Alaerts, N. Matthijs, Methodology to develop liquid chromatographic fingerprints for the quality control of herbal medicines, Acta Chromatographica – in press

In preparation

S.Pieters, C. Tistaert, K. Bodzioch, B. Dejaegher, D. Mangelings, V. Nguyen Thi Hong, C. Rivière, M. Chau Van, J. Quetin-Leclerq, Y. Vander Heyden, Evaluation of pressurized capillary electrochromatography in a screening for possible antioxidant molecules in *Mallotus* fingerprints using partial least squares regression models: Challenges, potentials, and prospects

G. Alaerts , B. Dejaegher, M. Dumarey, M. Merino-Areval, N. Noppe, N. Matthijs, J. Smeyers-Verbeke, Y. Vander Heyden, Chemometric exploration of chromatographic fingerprint to distinghuish *Rhizoma Chuanxiong* and *Rhizoma Ligustici*

G. Alaerts, B. Dejaegher, M. Merino-Aréval, L. Schockaert, J. Smeyers-Verbeke, Y. Vander Heyden, Distinguishing *Artemisia vulgaris* from related *Artemisa* species by means of chromatographic fingerprints measured at common conditions

C. Tistaert, B. Dejaegher, L. Angenot, J. Crommen, Y. Vander Heyden, Chemometrical treatment of fingerprints as a tool for quality control of *Citri reticulatae pericarpium*

Execution

Period: 01/12/2007 – 28/02/2010

Research Team/network:

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Discipline

Medicine/DRugs (pharmacopy)
Pharmaceutical Sciences