Macedonian pharmaceutical bulletin, 68 (Suppl 2) 201 -202 (2022)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.092
Short communication

Chromatographic fingerprinting for the detection of herbal adulteration and herbal fraud in plant food supplements

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Introduction

Plant food supplements (PFS) are gaining popularity in the western world due to the general trend of returning to natural and traditional products, but also the evolution towards self-medication, the questioning of allopathic medicines and the misperception that "natural" and "herbal" stand synonym for "safe" play a role (Rocha et al, 2016).

The popularity of PFS resulted in the development of a market offering a wide range of products, representing high profits. Unfortunately, this made these products also vulnerable for fraud and adulteration, especially when purchased via internet, an interesting platform for the trade in fraudulent products (Mosihuzzaman and Choudhary, 2008). In this context two possible health threats may occur: (a) the PFS does not contain the plant or herb declared on the packaging. This can be either due to falsification or fraud, or confounding. (b) adulteration, with the distinction between chemical adulteration and herbal adulteration. In the first case the PFS contains an active pharmaceutical ingredient (API), not claimed on the package, representing a serious health risk. In the second case the product contains active plants or herbs which are not claimed on the packaging, and are regulated or toxic.

This paper deals with the problem of herbal adulteration and herbal fraud, i.e. the absence of the claimed medicinal plant. Polymeric Chain Reaction (PCR) analysis is the golden standard for the detection and identification of a specific plant, but in case of adulteration, complex mixtures or the necessity of screening for a series of plants it can become more tedious (Ichim, 2019). Here an approach is presented based on chromatographic fingerprinting and chemometrics to perform a targeted screening for three and two regulated plants, often found in PFS for potency enhancement and slimming respectively.

Materials and methods

Materials

Reference standards of *Aristolochia Fanghi* roots, *Ilex Paraguariensisis* leaves, *Epimedium Spp.* leaves, *Pausinystalia Yohimbe* bark and *Tribulus Terrestris* fruits were purchased from the American Herbal Pharmacopoeia (Scotts Valey, California, USA).

All samples used in this study where plant food supplements, previously analyzed in our laboratory for the presence of pharmaceutical ingredients.

Chromatographic fingerprints

Test samples were prepared as a series of triturations of the reference plants with, respectively, lactose and six herbal matrices (negative PFS).

All chromatographic fingerprints were recorded using a Diode Array Detector at wavelength 254 nm.

To record the fingerprints, the extraction solvents and the chromatographic parameters were optimized to obtain a specific fingerprint for each of the targeted plants (Custers et al.,2017; Deconinck et al.,2019)

The real samples were divided into two groups according to their indication.

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After the screening, the results were confirmed using mass spectrometry (MS) by comparing the presence of identical signals in sample and reference, based on retention times and MS^2 spectra.

Data processing and modelling

The recorded fingerprints were used as such to create binary models. This means that for each targeted plant a model is created to distinguish between positive and negative samples.

The models are constructed using the triturations. Duplex algorithm was used to select a test set in order to validate the models. The best performing binary models were obtained with k-Nearest Neighbours, except for *ilex paraguariensis*, for which the model obtained with partial least squares-discriminant analysis was used.

Results and discussion

For each of the five targeted plants a specific chromatographic fingerprint was developed. The obtained method was used to record the fingerprints for the pure plant, the blank matrices, the triturations and the real samples.

The chromatographic fingerprints recorded for the plant references and the triturations were then used to build and validate a binary model, capable of distinguishing positive and negative samples for each of the targeted plants.

The validated models for *Aristolochia Fanghi* and *Ilex Paraguariensisis* were consequently used for targeted screening of 35 PFS with slimming as indication. In the same way the 34 samples classified as "potency enhancers" were screened for *Epimedium Spp, Pausinystalia Yohimbe and Tribulus Terrestris* using the respective models.

The screening resulted in one of the 35 slimming aids testing positive for *Aristolochia Fanghi* and 11 for *Ilex paraguariensis.* The former plant is forbidden in PFS, due to its nephrotoxicity. The latter is allowed, but should be labelled on the packaging, however none of the 11 positive samples claimed its presence on their packaging. Of the 34 potency enhancers the screening revealed one sample positive for *Epimedium spp.*, six for *Pausinystalia Yohimbe* and two for *Tribulus Terrestris*. Apart from *Pausinystalia Yohimbe* (forbidden), these plants have to be notified and claimed in the ingredient list. Three of the six samples found positive for Pausinystalia Yohimbe, claimed its presence on the packaging. Except of these three, none of the samples in the set claimed it. The sample found positive for Tribulus Terrestris mentions it, though 4 other samples claimed its presence, but were found to be negative based on the screening approach (Deconinck et al.,2019).

Except for one sample found positive for *Ilex paraguariensis* and the sample found positive for *Epimedium spp*. these results were confirmed using mass spectrometry detection.

Conclusion

This paper presents an approach to screen PFS for the presence of toxic and/or regulated plants based on a combination of chromatographic fingerprinting and chemometric modelling.

From a technical point of view the screening approach performs very well, though a small number of false negatives were detected during validation of the models, pointing at some lack of specificity. Off course errors are part of any modelling approach, but some possibilities exist to improve this specificity. The first is the systematic inclusion of samples in the models to incorporate matrix variability. A second is to increase the specificity of the fingerprints by using ultra high pressure liquid chromatography and taking more detection wavelengths into account. Preliminary results in this context showed promising results. Also the use of MS detection to record fingerprints could be explored.

The results of a small survey of suspicious plant food supplements proved that herbal adulteration and herbal fraud with plant food supplements is a real issue and that surveillance by competent authorities is necessary, especially for products purchased via Internet. It also showed that controls should not be limited to chemical adulteration, but also include its herbal version.

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