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Fuzzy clustering evaluation of the discrimination power of UV–Vis and (±) ESI-MS detection system in individual or coupled RPLC for characterization of *Ginkgo Biloba* standardized extracts

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ABSTRACT

Aim: Discrimination power evaluation of UV–Vis and (±) electrospray ionization/mass spectrometric techniques, (ESI-MS) individually considered or coupled as detectors to reversed phase liquid chromatography (RPLC) in the characterization of *Ginkgo Biloba* standardized extracts, is used in herbal medicines and/or dietary supplements with the help of Fuzzy hierarchical clustering (FHC).
Experimental: Seventeen batches of *Ginkgo Biloba* commercially available standardized extracts from seven manufacturers were measured during experiments. All extracts were within the criteria of the official monograph dedicated to dried refined and quantified *Ginkgo* extracts, in the European Pharmacopoeia. UV–Vis and (±) ESI-MS spectra of the bulk standardized extracts in methanol were acquired. Additionally, an RPLC separation based on a simple gradient elution profile was applied to the standardized extracts. Detection was made through monitoring UV absorption at 220 nm wavelength or the total ion current (TIC) produced through (±) ESI-MS analysis. FHC was applied to raw, centered and scaled data sets, for evaluating the discrimination power of the method with respect to the origins of the extracts and to the batch to batch variability.
Results: The discrimination power increases with the increase of the intrinsic selectivity of the spectral technique being used: UV–Vis < MS(−) < MS(+), but it is strongly sensitive to chemometric transformation of data. Comparison with cluster analysis (CA) and principal components analysis (PCA) indicates that the FHC algorithm produces better classification. However, PCA and CA may be successfully applied to discriminate between the manufacturing sources of the standardized extracts, and at some extent, to monitor the inter-batch variability. Although the chromatographic dimension sensibly contributes to the discrimination power, spectral MS data may be used as the lone powerful holistic alternative in characterization of standardized *Ginkgo Biloba* extracts.

1. Introduction

Nowadays fingerprinting and pattern recognition algorithms represent valuable tools for the characterization of the complex chemical mixtures of natural origins, with promising results in various application fields such as food [1,2], beverages [3,4], agriculture [5,6], chemotaxonomy [7,8], herbal medicines [9], dietary supplements [10,11], metabolic profiling [12–14], environmental [15,16] and standardization [17]. The European Medicines Agency [18] recommends that the appropriate fingerprinting procedures should be based on chromatographic techniques. However, other techniques, such as the spectral ones, may lead to interesting and useful results [19] as the US Food and Drug Administration [20] recommends.

Evolvement of fingerprinting procedures during the last decade has been supported by the application of powerful and advanced chemometric methods, among which principal component analysis (PCA), partial least squares (PLS), cluster analysis (CA), linear discriminant analysis (LDA) and artificial neuronal networks (ANN)

Abbreviations: ANN, artificial neuronal network; a.m.u., atomic mass unit; CA, cluster analysis; DAD, diode array detection; (±)ESI, positive/negative electro spray ion source; FHC, Fuzzy hierarchical clustering; HPLC, high pressure liquid chromatography; IBJ, Backer–Jain Index; LDA, linear discriminant analysis; mAU, milli-absorption unit; MS, mass spectrometry; PCA, principal components analysis; PCN, normalized partition coefficient; PEN, normalized partition entropy; PLC, partial least squares; RAM, random access memory; RPLC, reversed phase liquid chromatography; TIC, total ion current; TOC, total organic carbon; UV-Vis, ultra-violet and visible

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comparing to RPLC/(+)MS data. This remains valid for both raw and scaled values.

For informational purpose, projection of all samples in the space defined by the membership degrees to various clusters obtained from raw and scaled data sets is presented in the Supplementary material: Parts D and E, respectively. According to the technique of investigation, classification power varies for raw data in the order UV < RPLC/(−)MS < RPLC/UV ≈ (−)MS < RPLC/(+)MS ≈ (+)MS, while for the scaled data the succession differs slightly: UV ≈ RPLC/(−)MS ≈ RPLC/(+)MS < RPLC/UV < (−)MS < (+)MS.

3.3. Comparison of FHC to PCA and CA results

The discrimination power provided by FHC may be also evaluated through comparison to results produced by the common classification methods, more specifically PCA and CA. The PCA eigenvalues enlisted in Table 3 confirmed the FHC results, meaning that the hyphenation of MS to RPLC substantially favors discrimination power between samples.

Both processing algorithms behave similarly on the given data sets. Figs. 4 and 5 contain classifications produced through application of PCA and CA, respectively.

Both PCA and CA produced isolation of manufacturer A against the others when considering all data sets, except those originating from (−) MS and UV techniques. Exactly the same situation could be observed on grouping manufacturers C–F against A and B. Manufacturer B was identified based on data provided by all techniques when processed by PCA. Under CA processing algorithm, RPLC/UV and UV taken individually failed to discriminate between manufacturer B and the other ones. Succession within manufacturer's B batches was obtained only with PCA and CA, based on RPLC/(+)MS data set. Exclusion of the RPLC dimension made processing procedures insensitive to the batch production order.

PCA and CA did not allow identification of manufacturers E and F, as individual batches. PCA and CA failed also to classify manufacturers C and D, each containing two batches. Under PCA processing conditions the classification power varied in the order UV < (−)MS < (+)MS ≈ RPLC/UV < RPLC/(−)MS < RPLC/(+)MS. The order is slightly modified when data were treated through the CA algorithm: UV < (−)MS < RPLC/UV < (+) MS < RPLC/(−)MS < RPLC/(+)MS.

4. Conclusions

It appeared from the experimental approach that batch to batch discrimination may be achieved only through RPLC/(+)MS and FHC or CA analysis of data. Manufacturers A and B were easily identified by either HPLC/MS or MS approaches. With respect to the ability of discrimination between manufacturers C–F, the FHC approach is more powerful compared to PCA and CA. Data interpretation through PCA allowed the lower classification power against the used chemometric treatments. Within the FHC treatment, processing of raw and centered data produced similar classifications, while scaling data induced a negative effect on the discrimination capacity that derived from their gathering tendency.

As expected, the raw intrinsic information produced by the analytical investigation technique of samples plays an important role in tuning the discrimination power. The chromatographic dimension was necessary to observe the batch to batch variability. However, the chromatographic separation does not add significant insights with respect to manufacturer's identification. The holistic comparison of the classification ability of the analytical techniques used during the present work results in the following ranking order: UV < RPLC/UV < (−)MS < RPLC/(−)MS < (+)MS < RPLC/(+)MS. This hierarchy seems difficult to interpret at a first sight, as long as the number of peaks in chromatograms is relatively the same irrespective to the detection system that was used, while signals in the (+)/(−) mass spectra are identical. The last position occupied by the UV spectrometry can be easily explained, since differences between samples are based on relatively small shifts of the three or four existing absorption bands. The ability of the MS spectral investigation modes to overcome UV is also obvious. The selectivity induced by the RPLC separation is balanced by the ionization characteristics of the ESI and the intrinsic sensitivity of the MS detection. Compounds in samples simultaneously ionize and the mild ionization technique seriously limits the fragmentation of the produced molecular ions. This results in a limited overlapping of signals in the resulting spectra, while conserving the ability to consider most of the components from the complex mixture, including minor ones. Thus, the mass spectra become perfect carriers of subtle differences and the discrimination ability increases. The superiority of (+) MS over (−) MS in detection is far more difficult to explain since negative ionization generally occurs more selectively compared to the positive one. However, one should consider that the composition of the mobile phase was optimized for positive ionization. Consequently, (−) MS spectra exhibit signals having at least one order of magnitude lower intensity compared to (+) MS ones, and the ability of observing minor components decreases. The increased sensitivity in the positive ionization mode accounts for a specific pattern added particularly by these minor compounds in the samples and largely contributes to the increase of the differentiation ability.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.035.

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