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Comprehensive Analysis of Phytopharmaceutical Formulations – An Emphasis on Two-Dimensional Liquid Chromatography

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Abstract

In spite of the efforts in increasing the analytical control on herbal medicines, difficulties in the standardization of their components and the absence of a thorough characterization of the chemical composition still persist and constitute obstacles to the safe therapeutic use of these substances. The public consider these products inoffensive and uses them intensively or extensively, including specific groups of population like children and elder people. Thus, it is important to know as much as possible the chemical composition of these phytopharmaceutical products and quantify their pharmacologically active components. The analysis of plant extracts is a difficult task, as they are usually very complex mixtures. The separation power of traditional one-dimensional methodologies is generally inadequate, so two-dimensional separation is required to provide enhanced separation, since it enables to achieve higher overall peak capacities. Furthermore, comprehensive 2D analysis can provide a fingerprinting analysis of samples, allowing the detection of differences in the presence and/or concentration of compounds in complex mixtures.

Keywords: Phytopharmaceutical formulations; Comprehensive analysis; Fingerprinting; Two-dimensional liquid chromatography

Introduction

One-dimensional (1D) separation techniques, such as liquid and gas chromatography and electrophoresis have emerged in the last century, and their sustained development since then has demonstrated their reliability, robustness and broad field of application in Analytical Chemistry. However, as they became mature separation techniques, new challenges were faced by analytical chemists, in what concerns the analysis of more and more complex samples and the need to obtain complete information about sample composition, along with detection and quantification of small differences between samples.

Nowadays, the requirement of a full characterization of samples is observed in a variety of areas, namely food [1,2] and environmental [3,4] control, clinical monitorization [5], drug and biomarker discovery [6,7].

To face these demands, comprehensive separation approaches have been developed, in which all components of the sample are submitted to the separation process. The need to analyze complex samples with hundreds of constituents called for separation systems with larger peak capacities, that is to say, with the ability to separate, with an adequate resolution, a high number of peaks between the first and the last eluting peaks in a chromatogram. One of the ways of increasing the peak capacity is by increasing the separation space (between the first and the last eluting peaks). In order to achieve this, 1D separation has been replaced by multidimensional separation schemes, where two or more separation mechanisms are combined to enable a more efficient separation of the sample's components.

In a two-dimensional (2D) chromatographic system, the final peak capacity is the product of the individual peak capacities of each dimension [8,9]. Though, to attain this high peak capacity, two conditions must occur. First, the resolution obtained on the first dimension should not be lost on the second dimension, so back-mixing of separated peaks during the transference from the first to the second dimension should be avoided. According to Murphy et al. (1998) [10], this is achieved by performing three to four samplings per peak separated on the first dimension. For that reason, in liquid chromatography × liquid chromatography (LC × LC) modes, the first separation is performed along the dimension that demands the widest space, thus the longest time. Second, the mechanisms used to separate the compounds should be preferably independent (orthogonal). In practice, perfect orthogonal combinations are very difficult to reach, since retention of analytes depends on their physical and chemical properties, which are often correlated. For example, the molecular size of peptides correlates strongly with their hydrophobicity and net charge, thus their separation by ion exchange chromatography on the first dimension, followed by reverse phase liquid chromatography (RP-LC) on the second dimension, will not be completely orthogonal. Therefore, the most orthogonal combination of separation mechanisms is always dependent on the sample's composition and its dimensionality, which is defined as the number of parameters (dimensions) that are necessary to account for the chromatographic properties of its components [11]. In the limit situation, if the two separation mechanisms are the same, the peak capacity will correspond to the sum of the individual peak capacities of each separation [8,9].

The advantages of 2D separations over 1D were already described in the 1970s, by [12,13], for the analysis of complex samples. A significant increase in the number of compounds was obtained if two sufficient orthogonal separation mechanisms were used in the analysis of proteins or plant extracts, compared to 1D separation.

In recent years, several forms of 2D liquid phase separation methods became the basic support in proteomics research [14-21].

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Two-dimensional separations have also found application in the metabolomics field, in areas like clinical diagnosis and monitoring, toxicity assessment and nutrigenomics [22,23]. Samples derived from proteomic and metabolomic research are highly complex, thus requiring multidimensional separations for the successful resolution of a huge number of components, present at widely distinct concentrations in the referred samples. Also in the pharmaceutical field, multidimensional separations are required for the identification and quantification of drugs and their metabolites in biological fluids such as plasma or urine [24-28] and for impurity detection and identification as a quality control in drug substances and final products [29,30].

Because of its high resolving power, two-dimensional liquid chromatography (2D-LC) has been one of the most reported and implemented approaches in the comprehensive analysis of complex samples, and where new methodological approaches were developed.

Comprehensive Analysis of Herbal Medicines

The use of herbal medicines

Traditional herbal medicines (THM) have been used for centuries, by billions of people worldwide, in the prevention and treatment of human diseases. WHO estimates that around 80% of the population in developing countries uses traditional medical practices as a primary approach to their health problems [31]. A high percentage of people living in industrialized countries also choose to do so and, in the recent decades, the use of phytopharmaceutical products has been increasing in the developed countries. WHO estimates that over 100 million Europeans are currently users of traditional and complementary medicine (T&CM), with one fifth regularly using these products or practices, and the same number preferring health care which includes T&CM (European Information Centre for Complementary and Alternative Medicine). There are many more T&CM users in Africa, Asia, Australia and North America [32]. This behavior does not imply a break up with conventional medicine, but frequently people use natural products concomitantly with conventional pharmaceuticals. Supplements for obesity control represent one of the phytotherapeutic products most used by population, though some of the substances included in these products are associated with adverse effects [33]. The use of phytotherapeutic products is also increasing in pregnant women, as food supplements [34] and in children [35].

So, the patterns of use of THM vary among countries, and depend on factors such as culture, historical significance and regulations. In some countries, the availability and/or accessibility of conventional health care products is limited, thus people use THM as primarily source of health treatments. This is observed in Africa and some developing countries [36]. People may also use THM due to culture and historical influences. In some Asian countries, where the conventional health system is well-established, the majority of the population commonly uses THM [37]. Finally, people use THM as complementary therapy. This is what happens typically in developed countries, in Europe and North America.

Besides common drives to use THM, other individual reasons underlay its utilization, namely an increased demand for all health services, a desire for more information leading to an increased awareness of available options, an increasing dissatisfaction with existing healthcare services, and a rekindled interest in "whole person care" and disease prevention which are more often associated with T&CM. In addition, T&CM recognizes the need to focus on quality of life when a cure is not possible [38]. In general, there has been an increase in self-healthcare as consumers choose to be more proactive about their own health and people usually have the perception that "natural means safe" (which is not necessarily true) [39].

Regulation and guidelines for quality control of herbal medicines

Most countries have different ways of defining herbal medicines and adopted diverse approaches for licensing, dispensing, manufacturing and trading these products. The situation is more precarious in many developing countries where, despite the large use of THM and empirical knowledge about them, there are very few legislative guidelines to incorporate these products in national drug policies [40]. In fact, some accidents have been reported, associated with the use of herbal formulations, which reflects a lack of attention in this area and/or insufficient research [41].

Therefore, considering the need to assure the quality, safety and efficacy of herbal medicines both in industrialized and developing countries, and in face of the growing use of these products, several official entities have been emitting legislation to regulate the production and quality control of these products [42-47]. According to the legislation, the presentation dossier of these products must include a description of all the components with known therapeutic activity (with molecular and structural formulas, including stereochemistry) as well as the other components. WHO published a series of monographs whose purpose is to provide accurate scientific information on the safety, efficacy and quality control/quality assurance of widely used medicinal plants, and to assist the Member States to develop their own medicinal plant monographs [46]. The European, United States and China Pharmacopoeias have also published monographs on the quality control of herbal materials.

The majority of natural products used for therapeutic purposes is composed by one plant species or by complex mixtures of plants, presented in a natural or in a pharmaceutical form. The tendency is to use standardized plant extracts in the formulation, obtained by a patented extraction process. In either way, they should comply with the requirements that are normally specified in pharmacopoeias. These include assays for standardization of raw materials, such as organoleptic evaluation, micro and macroscopic examination, and assays to identify or to determine a representative profile of the main active constituents of the product, and also to quantify these compounds. Usually, these assays are performed by HPLC. In addition, elaboration of pharmacological, clinical and toxicological assays is intended to assure the three basic parameters that all pharmaceutical products must follow: quality, efficacy and safety.

In spite of the efforts in increasing the control on herbal medicines, their safety remains a problem. First of all, a difficulty in the standardization of their components constitutes an obstacle to the safe therapeutic use of these substances [48]. The chemical composition of herbal formulations depends on several factors, such as climate parameters, botanical species, cultivation conditions, harvest time, anatomical part of the plant used, storage conditions and extraction procedure [41,49]. Additionally, many plants used in phytotherapy don't have their constituents thoroughly characterized under the chemical and pharmaceutical point of view [50]. The majority of plants contain several substances with potential pharmacological activity, which difficult the exact identification of the constituent responsible for the therapeutic or adverse effect [51]. Not rarely, the active compound indicated in the product container is absent in the product itself [52]. By comprising pharmacologically active substances in their composition, they have the potential to induce adverse effects.

Quantitative discrepancies between lots and between similar products can be a source of adverse effects, since the difference between safety and toxicity is often dose-dependent [53]. Also, as they are frequently used along with conventional medicines, interactions may occur, enhancing or diminishing the therapeutic effect of the synthetic drugs. During their production, herbal medicines may suffer adulterations, for example, by addition of potentially toxic, unrelated substances [54,55]. Furthermore, the public consider these products inoffensive, as they are produced from a natural source and are easily reachable without prescription, and uses them intensively or extensively. Their use by specific groups of population, like children and elder people, with sub-optimum metabolism and excretion mechanisms, increases the occurrence of adverse effects and drug interactions. Lastly, the massive use of herbal medicines by population expands these concerns. Hence, for the referred reasons, it is important to know as much as possible the chemical composition of these therapeutic products and quantify their active compounds.

Fingerprinting techniques

Among all the procedures available to perform the quality control of herbal medicines, WHO recommends the use of chromatographic fingerprinting techniques [56]. Other entities, namely the European Medicines Agency [57], the Food and Drug Administration of United States [58] and the State Food and Drug Administration of China [59,60] have also accepted fingerprints as adequate techniques to evaluate the quality of herbal formulations. A fingerprint is a characteristic profile of a sample, which represents its chemical composition in a qualitative and quantitative way. Generally, fingerprints can be obtained using several techniques (chromatographic, electrophoretic and spectroscopic) [41,49,61-63], although the chromatographic ones are more frequently applied. In such cases, the entire chromatogram is the fingerprint and the analysis must fulfil some requirements: (a) a high peak capacity, since all components in the sample are potentially relevant; (b) retention-time stability and (c) detector stability, as recording the chromatograms may take considerable time; (d) a wide dynamic range, because both major and minor components are important and (e) the use of multivariate-analysis techniques (for example partial least squares or principal components regression), to correlate fingerprints with the product specifications and characteristics. The result of a fingerprint analysis may be a collection of chromatograms, a classification of samples or a set of peaks that correlates with a product property [64].

Chromatographic techniques used to obtain fingerprints include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC), hydrophilic interaction liquid chromatography (HILIC) and gas chromatography (GC).

TLC has the main advantages of readily available technique, easy to implement in the laboratory and flexibility to optimize working parameters, which makes it a good technique for fast screening analysis. On the other hand, its lack of reproducibility and resolution, high compound concentration requirements and the semi-quantitative nature of the technique disables its widespread use for herbal formulations fingerprinting [49]. Nevertheless, some methods have been developed for herbal analysis. For example, a 2D-TLC was developed to discriminate several varieties of *Heracleum spp.* [65]. A review on 2D-TLC in the analysis of secondary plant metabolites illustrates the advantages and drawbacks of using this type of comprehensive chromatographic separation in the analysis of natural compounds [66].

HPLC is the most used technique for herbal products characterization. Its high sensitivity, resolution, automation and the ability to couple the technique with different detectors explains its wide use. For herbal fingerprinting, several methods have been described and reviewed [49,67-71]. Main disadvantages of HPLC rely on the expensive machinery required and large volumes of environmentally unfriendly solvents used, undetected co-eluted compounds and the vulnerability of conventional silica-based columns to extreme mobile phase pHs and high temperatures [49].

The ability of UHPLC to perform fast and high resolution separations, better than HPLC, due to the use of smaller solid phase particles that allow the application of higher pressures, are making this a valuable and emerging technique for herbal medicines analysis. Comparing the results obtained with HPLC and UHPLC for the same samples, there is a reduction in analysis time and an enhancement in selectivity [72-77].

HILIC has demonstrated good performance in herbal fingerprinting due to its good retention and separation of hydrophilic and polar compounds with aqueous and polar organic mobile phases, which are more environmentally friendly compared to normal-phase liquid chromatography. Some applications for herbal products have been reported, associating HILIC and reverse-phase liquid chromatography to create orthogonality and, therefore, improve the separation capacity of the method [78,79].

GC presents high separation efficiency and sensitivity, adequate for the analysis of complex samples. Wang et al. [80] reported the separation of ephedrin-type alkaloids and their enantiomers in raw herbs and commercial herbal products by comprehensive GC × GC analysis. The proposed method showed improved performance, compared to single column GC analysis, providing adequate resolution in the separation of the alkaloids of interest, as well as from potential interference species in the sample matrix. The direct qualification and quantification of the volatile components of *Teucrium chamaedrys* was described by using a comprehensive 2D-GC – time-of-flight mass spectrometry (GC x GC–TOF/MS) system [81]. The GC x GC separation resolved hundreds of components within the sample, and with the separation coupled with TOF/MS for detection, high probability identifications were made for 68 compounds. In spite of the enhanced performance of 2D-GC, the use of high temperatures limits its application in the analysis of herbal formulations to essential oils, whereas the possible degradation of thermo-labile compounds and the necessary volatility of molecules difficult a wider use of the technique, when derivatization is not possible [82-85].

Recently, and to overcome some of the limitations in the conventional chromatographic techniques, there is a trend for miniaturized separation systems, where solvent consumption can be reduced, lessening the impact in environment. Nonetheless, a common drawback in these systems is the limited sample volume that can be introduced, which calls for additional preconcentration steps, increasing the complexity of the procedure and the analysis time [86,87]. The main technique used in miniaturized systems for the fingerprinting herbal formulations is capillary electrophoresis (CE) [88,89]. However, some drawbacks limit its use, namely the occurrence of overlapping peaks in complex samples and some irreproducibility in migration times due to fluctuations in electro-osmotic flow. In order to improve the performance of CE, capillary electrochromatography has been applied in the analysis of herbal samples, combining attractive features from CE and chromatographic techniques. This results in a high-resolution, selective and reproducible technique, adequate

for herbal fingerprinting [90-93]. Some difficulties in the use of miniaturized separation systems still remain, such as high backpressure of the columns aggravated by the viscosity of solvents, additional clean-up steps required for samples and low sensitivity, which impairs its wide use in herbal preparation analysis [49].

The need for a comprehensive analysis

Developments in Analytical Chemistry, namely in modern chromatographic, spectrometric and radioimmunological methods allowed to amplify the knowledge on herbal medicines, regarding their chemical composition and the structures of their active components. As a consequence, there is a better quality control of these products. Nevertheless, in the immense plethora of herbal medicines accessible to the public, not all products are controlled as they should be [48]. Besides, even the phytotherapeutic products that comply with all legal requirements should benefit from a more exhaustive analysis of their chemical content, in order to obtain a more complete characterization of their pharmacological profile.

In this scenario, comprehensive analysis provides a useful tool to perform complete characterization of herbal medicines. Unlike the analysis of conventional pharmaceuticals, where the identification and quantification of the synthetic active constituent is relatively easy, the analysis of plant extracts is a difficult task, as they are usually very complex mixtures. The separation power of traditional 1D methodologies is usually insufficient for separation of more complex samples and, in such cases, two-dimensional chromatography provides enhanced separation, due to their higher overall peak capacity. Furthermore, 2D analysis can provide a fingerprinting analysis of samples, allowing the detection of differences in the presence and/or the concentration of components in complex mixtures. This way, comparison between products of similar nature but different origins or sources can be performed, as well as the evaluation if a manufactured product is off specifications.

The concept of LC × LC was introduced by Erni and Frei [13] and it is based on the use of two chromatographic columns in series. These columns separate compounds in a sample according to two distinct characteristics. Between the first and the second columns there is a modulator that continuously traps small portions of the effluent from the first column and releases them on the second column (Figure 1).

The result is a very detailed 2D-chromatogram presented in three dimensions (two retention time axes and an intensity axis). This form of operation enables an increase in the peak capacity of the whole separation. Theoretically, the total peak capacity equals the peak capacities of the two individual dimensions [94]. Fingerprints obtained with two-dimensional LC surpassed 1D chromatography fingerprints, multidimensional fingerprints generated by hyphenated techniques and multiple fingerprints, and the main disadvantage of the technique is the time needed to perform a full separation [69]. Comprehensive two-dimensional liquid chromatography has been thoroughly reviewed [67-69,95,96].

Typically, phytopharmaceutical compounds are separated by reverse phase (RP) LC. Furthermore, RP columns are generally used in second dimension separations, due to their compatibility with MS. Nevertheless, combinations of normal phase (NP) × RP, HILIC × RP and RP × RP may be used. NP × RP is the combination with the theoretically highest degree of orthogonality, but solvent incompatibility is a serious limitation. Even so, it can be applied if a first dimension column with a small diameter relative to the second dimension column diameter is used [97], reducing the volume of effluent to be injected on the second column, but also reducing analytical sensitivity. Alternatively, the addition of water to NP solvents may increase their miscibility with RP solvents. HILIC x RP is also a highly orthogonal combination [98]. HILIC uses a polar stationary phase and a water gradient (the mobile phase is what distinguishes HILIC from NP chromatography). The usefulness of this combination was already demonstrated in proteomic separations [99]. Although its application has some difficulties, namely incompatibility of the solvents used in HILIC and RP, impairing their on-line coupling, several improvements have been reported to surpass this limitation [100,101]. As for RP × RP, it has the broadest application, enables gradient elution on both dimensions with fast speed and high peak capacity, but orthogonality strongly depends on the column or mobile phase choice. RP columns need to be prepared with different functionalities in order to make them more orthogonal among themselves. Changing the pH of the mobile phase can also induce drastic alterations of the separation selectivity of RP columns [102]. To reduce time analysis in second dimension and, thus, faster transferences, monolithic columns may also be used. Other parameters to be optimized in method development are the flow rates used in both separations and temperature. Second dimension separation is the rate controlling step in 2D-LC, thus several approaches were reported to speed it up. The use of supra-ambient temperatures on the second dimension separation is one of the possibilities to speed up gradient elution separations. The eluent viscosity decreases with increasing temperatures, enabling the use of higher flow rates at the same pressure. This results in fast and reproducible gradient elutions and a fast column reconditioning [103], thereby shortening the overall comprehensive separation. There is the fear that the use of higher temperatures may induce analyte decomposition, but some studies have shown that even thermally labile pharmaceuticals and peptides can be analyzed with little evidence of decomposition [104].

The choice of eluents has to follow the requirements of on-line LC × LC separations: the eluent used in first dimension must be a weak eluent in the second dimension, both eluents must be miscible and salt precipitation should not occur. The gradients to be used must be optimized, aiming the best compromise between resolution and analysis time.

In sample preparation, if required, extraction techniques such as solid-phase extraction may be applied. On-line extraction is sometimes used, since it enables higher recoveries, as reported [105-109].
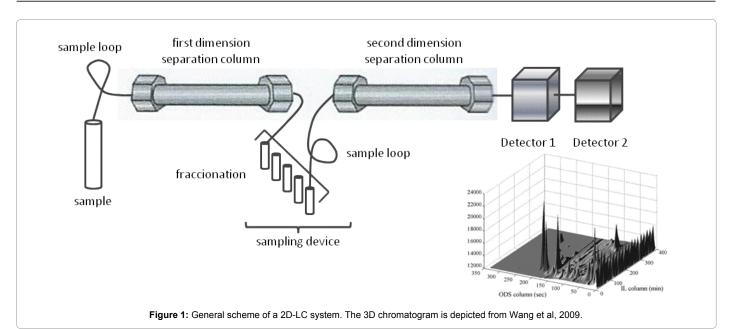
Method optimization must contemplate the automation level, considering the application of the developed methodologies in routine analysis. Therefore, the possibility to perform the method procedures on-line, namely sample preparation and cleaning/regeneration of equipment between samples should be explored.

In order to reduce reagent consumption and waste production, a tendency to miniaturize the manifolds has been followed.

Since the optimization of the chromatographic method is a complex task due to the number of parameters affecting the separation, chemometric methods ought to be used, such as factorial design. This approach enables to obtain the combination of variables which provides the best analytical response with a limited number of experiments [110].

Analytical applications of 2D-liquid chromatography in the analysis of phytotherapeutic products

LC x LC techniques have been applied to the analysis of phytotherapeutic products (Table 1). A 2D-LC system with an

Figure 1: General scheme of a 2D-LC system. The 3D chromatogram is depicted from Wang et al, 2009.

Herbal formulation	Column features	Resolution	Reference
Longdan Xiegan Decoction	1st column: immobilized liposomes 2nd column: RP	more than 50 compounds were separated	Wang et al., 2009
Rhizoma chuanxiong	1st column: cyano 2nd column: ODS	more than 50 compounds were separated	Chen et al., 2004
Saponins in extracts of *Panax notoginseng*	1st column: HILIC 2nd column: RP	224 saponins were found (peak capacity 10200)	Xing et al., 2012
Extracts of umbelliferae herbs *Ligusticum chuanxiong Hort* and *Angelica sinensis (Oliv.) Diels*	1st column: cyano 2nd column: silica monolithic ODS	between 100 and 120 components were efficiently separated in each extract	Hu et al., 2005
Flavonol glycosides in the leaves of *Maytenus ilicifolia*	1st column: size exclusion 2nd column: RP	better resolution compared to 1D analysis	Souza et al., 2009
Extracts of *Psoralea corylifolia*	1st column: ion exchange 2nd column: RP	more than 188 components were separated	Chen et al., 2005
Extract of *Rheum palmatum L.*	1st column: silica-bonded human serum albumin 2nd column: silica monolithic ODS	better resolution compared to 1D analysis	Hu et al., 2006

Table 1: Applications of 2D-LC analysis on the comprehensive characterization of herbal formulations

immobilized liposome chromatography column in conjunction with a RP column was developed for the screening and analysis of the membrane-permeable compounds in a traditional Chinese medicine (Longdan Xiegan Decoction) [111]. The authors reported that more than 50 components in the sample were separated using the developed separation system, which demonstrated to be useful in the identification of membrane permeable natural products in complex matrices such as extracts of traditional Chinese medicines. A different comprehensive 2D-LC separation system based on the combination of a cyano and an ODS column was developed for the separation of components in a traditional Chinese medicine (*Rhizoma chuanxiong*) [108]. More than 52 components in the extract were resolved and 11 of them were preliminary identified according to their UV and mass spectra. A comprehensive off-line 2D-LC method coupling HILIC and RPLC was developed for the detection of saponins in extracts of *Panax notoginseng*. The orthogonality of the 2D HILIC × RPLC was up to 81%, and the peak capacity was 10200. In total, 224 saponins were found, and some of them were trace amounts. In addition, a screening table designed by adding molecular weights of possible aglycones and sugars was constructed to help rapidly characterize the saponins using MS information [112]. Hu et al. [113] reported the use of a silica monolithic column in the second dimension separation, for the analysis

of two extracts of umbelliferae herbs. The system performance was superior to that using a conventional column in the second dimension, due to the increase of the elution speed on the second dimensional monolithic column. It was observed that much more peaks for low-abundant components in the herbal extracts could be clearly detected, compared to previous analysis. Between 100 and 120 components were efficiently separated in each extract in shorter analysis time. A 2D (size exclusion – reversed phase) system was developed to analyze flavonol glycosides in the leaves of *Maytenus ilicifolia,* commonly used in traditional Brazilian medicine [114]. The two-dimensional system showed better performance than 1D analysis and a greater number of compounds could be determined. The strategies used for the analysis of Chinese herbal medicines have been reviewed and include LC × LC, which demonstrated a powerful separation ability, high peak capacity and excellent detectability, compared to single dimension HPLC [86].

Also, there have been improvements in the development of novel 2D-LC systems, by using new interfaces between both columns in order to increase the number of fractions transferred from the first to the second column, reducing the risk to loose resolution of the primary dimension [62] and the application of monolithic columns for phytochemical analysis, which convey some advantages, namely

an increase in the flexibility of the technique regarding chemistries and functional compositions of stationary phases, low separation impedance, compatibility with micro and nanoformat separations, low time and labour consumption and cost-efficiency [115]. Monolithic columns are mechanically robust, may be prepared easily in situ and the porous properties can be controlled, its synthesis is flexible (monoliths can be modified with a broad range of functionalities), no void volumes are formed with conventional LC flow rates and the dominance of convection over diffusion in mass-transfer under dynamic conditions allow the use of higher flow rates to carry out the separation without loss of resolution. Since they need short conditioning times, they can be used in successive gradient runs. However, their use is limited by the commercially available column dimensions and they present a lower surface area, which means lower binding capacity [116].

Improvements in instrumentation used for sample preparation in the analysis of plants were also reported [107,109,117-124].

A number of chromatographic applications for the comprehensive analysis of herbal products were reported so far, but the detailed characterization of these formulations could benefit enormously from the implementation of two-dimensional LC since it could provide a sensitive characterization of extracts, in order to allow a chemical fingerprinting which globally addresses their chemical composition. Additionally, it could allow the simultaneous determination of the main compounds associated with therapeutic activity, for quality control, and the differentiation of plant species from different origins.

Conclusions

The small review herein exposed showed that 2D-LC is a very powerful separation technique, but yet not vastly applied in the comprehensive analysis of several complex matrices such as herbal medicines. This may be due to the highly sophisticated technique, the high costs and the requirements for trained personnel to work with this technique, besides the time needed to perform a complete fingerprint analysis. On the other hand, there is no doubt that the comprehensive analysis would benefit this research area and the implementation of two-dimensional-LC methods would greatly expand the analysis possibilities, both qualitative and quantitative, that the phytotherapeutic products, here mentioned, require. In the near future, as the technique becomes more widespread and applied, new applications in the analysis of these samples will surely be developed and reported.

Acknowledgements

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