

APPROACHES TO THE QUALITY CHARACTERISATIONS OF MEDICINAL PLANT DERIVATIVES

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Herbs versus synthetic drugs

With the exception of pure substances, botanical derivatives obtained from medicinal plants usually contain several classes of compounds endowed with a polyhedric mechanism of action which surprisingly often act synergistically on the same target. For these reasons some natural products, selected throughout history fit well with the multidrug approach. The cocktail of different compounds, which is normal in botanicals, seems to be increasingly useful for synthetic drugs as well, as is the case of the combination of cytotoxic compounds in cancer chemotherapy to overcome multidrug-resistance; the combination of several antiviral agents in AIDS therapy and the combination of different drugs in reducing the atherosclerosis damage.

However only few out of the several hundred extracts now used as herbal medicinal products, have documentation comparable to that of synthetic drugs; the majority are lacking good chemical characterisation, accurate drug master file documentation, toxicological investigation (teratogenesis, mutagenesis, fertility etc.) and controlled clinical trials. Very often, for the same plant, totally different extracts are present on the market claiming to possess the same activity and efficacy. Extracts derived from 1,350 plants are present today in formulations widely commercialised in the 8 most industrialised countries. They are mainly used for the treatment or prevention of mild pathologies or in combination with well established allopathic drugs to reduce their dosage and consequently some side effects.

Among these active ingredients, 202 are pure compounds, almost exclusively used as ethical drugs or as synthons for ethical drugs, whereas the others

are standardised extracts or extracts prepared according to the various Pharmacopoeias or Monographs established country by country. (Fig. 1)

Examples of such a distribution are given in Fig. 2, 3.

The list of the pure compounds includes all the well known alkaloids, terpenes, cardiac glycosides etc.(Fig. 2)

For these products the quality and the documentation criteria are established exactly as for the synthetic drug.

In Fig. 3 are reported the most important standardised extracts largely used as prescription drugs in many European Countries with an acceptable chemical, pharmacological and clinical profile. Apart from these standardised extracts as mentioned above, there is a plethora of other extracts currently used in many countries with old documentation or with the certification based only on the traditional use.

Usually, these extracts are present in multicomponent formulations, often registered at the beginning of this century, industrialising the galenic preparations of pharmacists behind the counter.

Quality standards

Many of these products represent the future basis for alternative or complementary medicine in later years. Of course given that the patients are not “alternative patients”, the quality of the products used as a substitute of drugs must comply with the safety and efficacy requirements for any modern formulation; above all the chemical composition of the active ingredients must be carefully checked if biological results are to be constant. (Fig. 4)

To get reproducible biological data in terms of safety and efficacy as mentioned above, the botanicals must first of all be standardised, to the active ingredients when they are known, or, to specific markers when the actives are not yet recognised.

As far as the chemical standardisation of botanical derivatives is concerned, on the industrial side the quality of the raw plant material is essential and this is the crucial point for the success of the preparation of any derived natural products.

The parameters influencing the quality of the plant material and as a consequence the standardisation grade of the extracts, are the following (Fig. 5):

In the selection of the biomass there are two possibilities: plants harvested in the wild or cultivated. The more complicated problem concerns the wild crop, which up to now has been the most frequent source. In this specific case some precautions must be kept in mind before proceeding with the extraction process (Fig. 6):

The most important points in using wildcrafted crops are the harvesting and drying conditions of the biomass; the traditional drying process of plant material in the shade or sunlight is usually very deleterious for the stability of the active principles and for the constancy of the biomass.

Nowadays many of these problems have been solved through the cultivation of genetically manipulated strains of plants or through cultivation in general, installing *in situ* all the facilities necessary to perform the best preparation of the biomass. Very often the drying process is pivotal. Cultivation is also necessary because wildcrafted crops of the widely used plants are no longer sufficient if the ecosystem is to be preserved; in addition the cultivated material may be harvested at the proper time and mechanically dried before the enzymatic and microbial degradation which often occur in the wild collection due to transportation problems or adverse climatic conditions.

As far as the chemical standardisation of the ex-

tracts is concerned, it is enough to say that plants normally contain several active substances in a certain ratio. In standardised extracts this ratio must be kept within narrow limits, constant from one preparation to another, which means starting from standardised biomasses.

Batch standardisation

To attain this first goal, quali-quantitative determinations of the active principles in the biomass must be done and at the extraction stage several batches must be combined to achieve a homogeneous mix; only with the combination of different batches, establishing a constant ratio among the main components, it is finally possible to get a product complying with the modern requirements for any drug. It is very important, in fixing the quality of the biomass, to include in the quality control a realistic average of the active ingredients; the tendency of the producers is to set this value as low as possible in order to have maximum acceptability of the crop, but this fact can create manufacturing problems for the reproducibility of the final product. On the other hand, when this value is too high the final product must be diluted with excipients and this is one of the reasons why there are present in the market completely different extracts under the same name.

For the preparation of a standardised extract of both wild or cultivated crops to standards set by international market regulatory authorities, the extraction solvents and the purification procedures should be carefully controlled during the manufacturing process to confer a defined chemical profile to the final extract.

As far as the chemical standardisation of such an extract is concerned, a fingerprint has to be established and a quantitative ratio among the various principles fixed; in other words it could be of interest, for instance, to fix a quantitative relationship between a couple of classes of compounds, the active principles and some characteristic products present in the extract. Linking two classes of compounds, the mathematical assurance of the perfect

reproducibility of any extract prepared with the same solvent can in effect be reached. Fixing a reasonable bracket of variation among the components and with the application of GMP procedures in the industrial preparation, we will obtain standardised extracts that can be documented with the safety and efficacy requirements demanded of any product for human use.

Standardised extract preparation

The preparation of highly standardised extracts, for the reasons above mentioned, is very often more complicated than the isolation of pure compounds. The preparation of such extracts involves common steps briefly summarised in Fig. 7.

Validated analytical methods applied to the drug remain the key point to get a standardised extract. Given that several components are present and that their stability, when isolated in pure form, is often poor, it is advisable as a general route in validation of the analytical methods, to select a single external standard and to establish their reciprocal ratio for quantitative evaluation.

Another aspect, very crucial for the extracts is the control of their stability. (Fig. 8)

Stability control

With botanicals there are two main problems: first, the stability of the active constituents and second the potential degradation of the unknown substances which very often represent the main part of any extracts. The former problem is solved using specific methods whereas the second could be solved using spectroscopic techniques, like NMR, which allow a global determination of all the components present in the extract.

On the practical point, I would like to give you few examples of standardised extracts largely used as a drug or health food and the techniques used for their characterisation following the criteria mentioned above. The extracts are those prepared from the fresh fruits of *Vaccinium myrtillus* (Myrtocyan[®]), from *Ginkgo biloba* leaves, from the seeds of *Vitis vinifera*

(LEUCOSELECT[™]) and from *Hypericum perforatum*.

Vaccinium myrtillus extract, which is largely used in ophthalmology in many European countries and in the United States, due to its antioxidant and free radical scavenging properties, is prepared from the fresh berries of the plant. The fruits contain as active components 15 anthocyanosides derived from five aglycones and having at C-3 alternatively three sugar units. The technology involved in the realisation of such an extract requires an alcoholic extraction of the anthocyanosides from the frozen blended berries to block the oxidase and hydrolase enzymes. The crude alcoholic extracts are concentrated to water and the anthocyanoside complex isolated using adsorption resins. After further purification to remove undesired substances the extract is concentrated to dryness. The process is monitored by a specific analytical method at every step in order to obtain an extract with the constancy required for a pharmaceutical product. This purified extract produced by Indena contains polyphenolic proanthocyanidins and 38% of active constituents, the anthocyanosides with a reproducible fingerprint and variations among the single components not exceeding 5%. (Fig. 9)

The fingerprint and the quantitative analysis are obtained by HPLC. (Fig. 10)

For the value of the active ingredients expressed in one of the major compounds a bracket of $\pm 2\%$ has been established

The *Vaccinium myrtillus* extract contains only one class of active principles, so the standardisation is not very complicated.

A different approach, due to the complexity and number of active principles, is required for instance in the case of *Ginkgo biloba* extract.

The leaves, which are the part of the plant medicinally used, contain 5 different classes of compounds all endowed with biological activity: flavonoids, diterpenes, sesquiterpenes, proanthocyanidins and

ginkgols. The chemical structures are very heterogeneous: (Fig. 11)

The preparation of this extract involves several purification steps after the extraction of the ground leaves with acetone/water 60% according to this scheme:

With this method the standardised extract contains the various active components in the following ratio:

The determination of these compounds in the extract is normally achieved combining HPLC and GLCMS techniques along with spectroscopic determinations for the substances not specifically determined. The ginkgoflavon-glycosides are evaluated by HPLC, the terpenes are evaluated by GLCMS, the proanthocyanidines by the Bate-Smith colorimetric reaction and ginkgols by HPLC. The flavonoids and terpenes fingerprints are reported in Figures 14 and 15.

What is important is the strict correlation among the different classes of compounds, each harmonised with the others for the final therapeutic goal. The extract used today in Europe is a perfect balance of the different fractions and any modification of this balance, e.g., any attempt to increase the content of any active principle which, tested separately, has displayed a marked specific activity, has turned out to be disadvantageous. To ensure the uniformity of the different batches and to check their stability, due to the complexity of the composition, we have introduced for the first time the ^{13}C -NMR determination in the quality control of the extracts.

Combining these techniques and fixing the ratio among the different classes of active principles, it is really possible to produce highly standardised extracts, which in terms of reproducibility can compete with pure pharmaceutical compounds, and to check their stability.

Another example of extract, widely used both in herbal medicinal products and in the health food market as an antioxidant or vasoprotector agent is represented by the standardised phenolic fraction

from grape seeds (LEUCOSELECT™).

The chemical characterisation of its active principles need a different technological approach. The extract prepared using a multistep procedure contains a homogeneous mixture of (+)-catechin and (-)-epicatechin oligomers randomly esterified with gallic acid. For the definition and standardisation of this extract two methods are used: HPLC and SEC chromatography. With these techniques it is possible to prepare a highly standardised extract which can comply with the requirements of a pharmaceutical product.

The last example of the chemical characterisation of botanical derivatives is the very popular extract of *Hypericum perforatum*. As the biological effects of *Hypericum* preparations are considered arising from the whole mixture of the main principles, the availability of a method allowing the analysis of the entire extract, rather than a single constituent, is desirable. An HPLC analytical method which allows the identification and quantitation of several compounds has been set up. This is the HPLC profile and all the components for their quantitative evaluations are expressed as rutin reference substance. Since availability and chemical stability of several of the constituents are critical, the use of the stable and easily available constituent rutin as an external standard has been proposed for the assay of the constituents of the extract.

Also for the *Hypericum* extract the ^{13}C -NMR can help in evaluation of reproducibility.

There are maybe other techniques for the evaluation of the extracts, like biological determinations of active components through affinity chromatography, receptor binding and so on but these approaches must be carefully investigated before a general application in the chemical characterisation of the extracts. However a biological evaluation in my opinion is necessary when important variations in process are performed in the preparation of extracts already used. Otherwise a big confusion remains among the many different products available to the consumer.