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7. High performance thin-layer chromatography (HPTLC) in the quality control of herbal products

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Abstract. The introduction of high performance thin-layer chromatography (HPTLC) for quality control of herbal products, using standardised methodology and system suitability tests for the qualification of the plates, has improved reproducibility. The use of intensity markers implemented by the Ph. Eur. improved the description and interpretation of the chromatograms. Quantitative information can be retrieved from the electronic images of the chromatograms and used for *comprehensive HPTLC fingerprinting*: a single HPTLC analysis gives information on identity, purity and content of an herbal drug/preparation/product, simplifying the quality control.

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Introduction

Herbal products are those that contain herbal drugs, herbal drug preparations or their combinations as active ingredients.

According to the European Pharmacopoeia (Ph. Eur.) [1], herbal drugs are mainly whole, fragmented or broken plants or parts of plants in an unprocessed state, usually in dried form but sometimes fresh. The word "plant" is here used in the broader sense to also include algae, fungi and lichens. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs.

Herbal drug preparations (or herbal preparations) are homogeneous products obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. They include, for example, extracts, essential oils, expressed juices, processed exudates, and herbal drugs that have been subjected to size reduction for specific applications, for example herbal drugs cut for herbal teas or powdered for encapsulation [1].

In most of the cases herbal products reach the market as medicines or as dietary supplements, depending on the region and the regulatory framework applied, which have different quality requirements. However, the quality of herbal drugs and herbal preparations is the basis for the reproducibility of the safety and the efficacy, and should be taken into account in all steps of the production process. The chemical complexity of herbal drugs and preparations, its variability inherent to its natural origin, the limited knowledge of the chemical constituents responsible of the therapeutic activity, and the possibility of adulterations and contaminations, make the quality control a challenging process [2].

The main objectives of quality control are to certify the identity of the herbal drug/preparation/product analysed, its purity concerning possible adulterations, falsifications and contaminants, and the strength/content of active principles or markers. Quality specifications published in Pharmacopoeias, which include analytical methods plus criteria of acceptance, are mandatory for medicinal products and can also be followed for dietary supplements [3].

Identification of herbal drugs and detection of possible adulterations rely in macroscopical and microscopical examinations and the analysis by thin-layer chromatography (TLC) or high performance thin-layer chromatography (HPTLC). In the case of herbal preparations, such as extracts, TLC/HPTLC are the preferred techniques and, sometimes, other chromatographic techniques, like gas chromatography (GC) in the case of essential oils, are used. Content/strength determination is done mainly by

high performance liquid chromatography (HPLC) and in certain cases by GC or spectrophotometry.

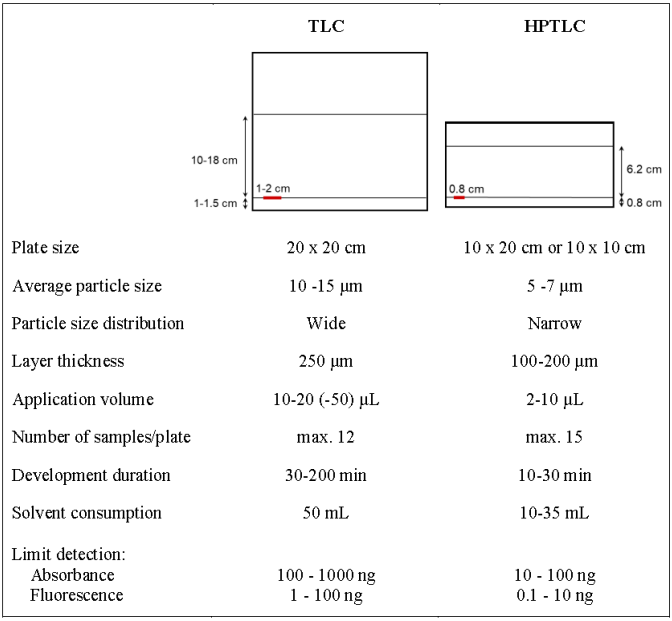
Although TLC was very early included in the Pharmacopoeias for the analysis of herbal drugs and preparations, significant improvements have been achieved by the introduction of HPTLC and the standardisation of the methodology. The objective of the present chapter is to introduce to the reader the new opportunities opened by these improvements that allow a deeper exploitation of HPTLC in the field of quality control of herbals.

1. High performance thin-layer chromatography

High performance thin-layer chromatography (HPTLC) is a planar chromatographic technique resulting from the evolution of the classical thin-layer chromatography (TLC), but it goes far beyond the old TLC performed on HPTLC plates [4]. Indeed, HPTLC and TLC share the same basic principles involved in the separation, which results from the interaction of the constituents of the sample with a planar stationary phase, the liquid mobile phase and the gas phase produced in the chromatographic chamber. Also, they share advantages such as visual results, parallel analysis of samples, single use of the plate, rapid results, flexibility and the possibility of multiple detection. Some technical differences between HPTLC and TLC, concerning the plate, solvent consumption, duration of development and sensitivity are shown in Fig. 1.

Beyond this differences and similarities, HPTLC is a new concept, which primary focuses are reproducibility and separation power. It uses well-defined methods, with optimized and standardized parameters, that pass a validation process, and give reliable analytical results with good intra- and inter-laboratory reproducibility. Instrumentation for HPTLC can be simple to sophisticated and allows obtaining traceable digital images and a deeper exploitation of the data contained in these images. Moreover, HPTLC is GMP friendly [4,5].

Well described sample preparation, well defined layout of the plate and volume of application, as well as fully specified chromatographic conditions and detection system(s), including not only the composition of the mobile phase, but the relative humidity, the use of a saturated, an unsaturated or a pre-conditioned chamber, the developing distance, etc. are necessary for reproducible results. The use of a method-specific system suitability test (SST) on each plate is a key point in order to qualify the developed plate, rely on each single analysis and compare analyses done on different plates.



	TLC	HPTLC
Plate size	20 x 20 cm	10 x 20 cm or 10 x 10 cm
Average particle size	10 -15 µm	5 -7 µm
Particle size distribution	Wide	Narrow
Layer thickness	250 µm	100-200 µm
Application volume	10-20 (-50) µL	2-10 µL
Number of samples/plate	max. 12	max. 15
Development duration	30-200 min	10-30 min
Solvent consumption	50 mL	10-35 mL
Limit detection:		
Absorbance	100 - 1000 ng	10 - 100 ng
Fluorescence	1 - 100 ng	0.1 - 10 ng

Figure 1. Some differential characteristics of TLC and HPTLC.

2. Quality of herbal medicinal products, HPTLC and Pharmacopoeia

The active ingredients (herbal drugs and herbal preparations, such as extracts or essential oils) of the herbal medicinal products must fulfill the quality requirements established in the corresponding regulations, including the related Pharmacopoeia. The objective of these specifications is to demonstrate identity, purity and content of active principles or markers [2,3].

TLC and, more recently, HPTLC has been traditionally used for proving identity of herbal drugs and extracts, as well as for the investigation of the presence of possible adulterations or falsifications, which is a key part of establishing the purity. In addition, HPTLC is also used, to a lesser extent, for the quantification of active principles or markers. The use of TLC/HPTLC for checking the identity and purity is mainly based on the visual observation of the chromatographic fingerprint, this is the sequence of zones of the chromatogram of the sample, taking in account the number, position (R_F) and color/fluorescence of the zones in one or more detection modes.

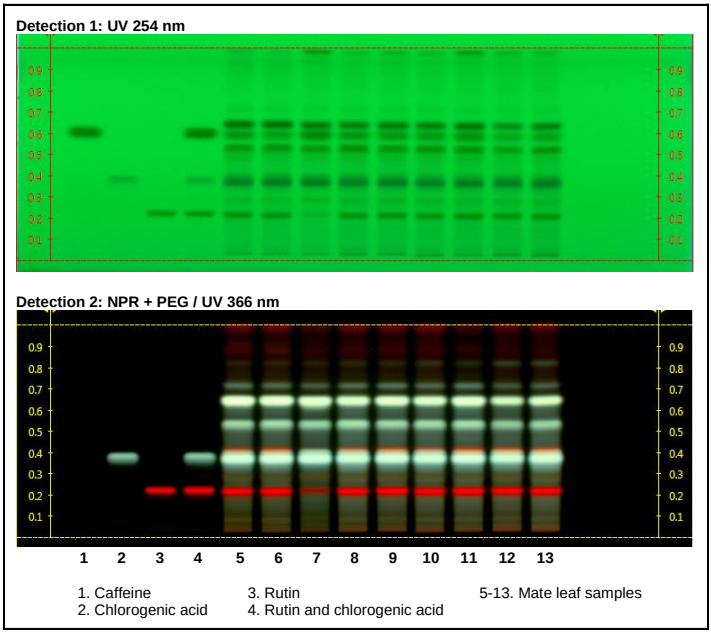


Figure 2. HPTLC chromatograms for identification of mate leaf (*Ilex paraguariensis* A.St.-Hil.) A single analysis with two subsequent detections allows the detection of caffeine, under UV 254 nm, and polyphenols (caffeoylquinic acids and flavonoids), under 366 nm after derivatisation with NPR and PEG. Developing solvent: toluene, water, anhydrous formic acid, ethyl formate, (3:6:8:60 V/V/V/V). Plate: HPTLC silicagel 60 F_{254}. NPR: Natural product reagent, PEG: Polyethylenglycol 400.

The possibility of multiple detection gives flexibility to TLC/HPTLC and allows detecting compounds of different phytochemical groups in a single analysis. Figure 2 shows, as example, the case of mate leaf (*Ilex paraguariensis* A.St.-Hil.), the characteristic constituents of which are caffeine and polyphenols, particularly caffeoylquinic acids and flavonoids [6]. In a single HPTLC analysis of the samples extracted with methanol, two detections are used subsequentially: first, the observation of the developed plate under UV light at 254 nm, allowing the detection of caffeine by comparison to a reference substance on a separate track, followed by a second detection using natural product reagent (NPR) and polyethylenglycol 400 (PEG) and observation under UV light at 366 nm. This second detection system cannot show the presence of caffeine, but detects a series of zones due to caffeoylquinic acids and flavonoids characteristic of mate leaf.

HPTLC is also very useful for discriminating close taxa that can be used as adulteration or falsification of the desired species. Recently, an optimized HPTLC method has been described that distinguishes the roots of 28 different *Angelica* taxa and related species belonging to the Apiaceae family, including those traditionally used in Europe (e.g. *Angelica archangelica* L., *Levisticum officinale* W.D.J.Koc) and those traditionally used in Asian countries such as *Angelica acutiloba* (Siebold & Zucc.) Kitag., *A. dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., *A. gigas* Nakai, *A. pubescens* Maxim., *A. sinensis* (Oliv.) Diels, *Ligusticum sinensis* Oliv., and *Ligusticum chuanxiong* S.H.Qiu, Y.Q.Zeng, K.Y.Pan, Y.C.Tang & J.M.Xu. [7].

Lavender flower (*Lavandula angustifolia* Mill.) is an herbal drug used mainly as sedative [8], whereas lavandin (*Lavandula* × *intermedia* Emeric ex Loisel.) is a hybrid between *L. angustifolia* Mill. and *L. latifolia* Medik., used for the production of essential oils as ingredients of industrial perfume and fragrance materials. The essential oils of both lavender flower and lavandin flower are characterized by a high content of linalool and linalyl acetate. Lavandin has higher biomass and oil yield, but the oil is considered of lower quality due to the higher content of 1,8-cineol and camphor [1,9].

The presence of a purple zone in lavadin flower, absent in lavender flower, allow an easy differentiation of both species by the HPTLC analysis of their toluene extracts, as it is shown in Fig. 3. In addition, the analysis clearly discriminates old lavender flowers from the herbal drug harvested in the current year.

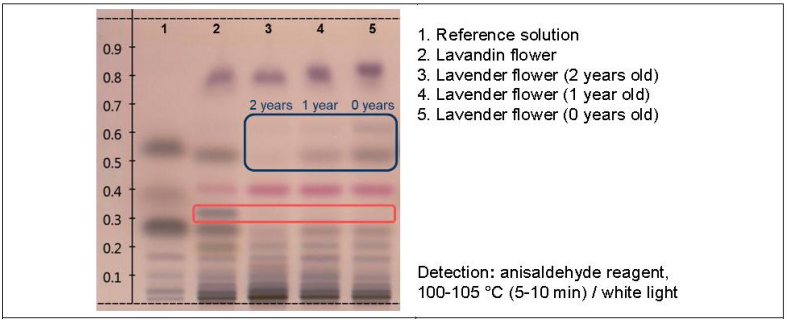


Figure 3. HPTLC chromatograms for identification of lavender flower (*Lavandula angustifolia* Mill.) and discrimination of lavandin flower (*Lavandula* × *intermedia* Emeric ex Loisel.) (see red rectangle). In addition, old samples (one and two years old) can also be distinguished from the sample of the current year (see blue rectangle). Developing solvent: ethyl acetate, toluene (5:95 V/V). Plate: HPTLC silicagel 60 F_{254}.

Gas chromatography (GC) is the technique of choice for essential oils, due to the volatility of their constituents, mainly mono- and sesquiterpenes. However, sometimes, essential oils are adulterated with fatty oils. Triglycerides, the major constituents of fatty oils are not volatile and, consequently, are not detected in the GC analysis of the essential oil: the GC profiles of the pure and adulterated essential oils are similar and the adulteration is not detected. HPTLC is able to distinguish between the pure and the adulterated oil and, in a second analysis, can give information on the fatty oil used as adulterant. As example, Figure 4 shows the case of the detection of this type of adulteration in the essential oil of sage (*Salvia officinalis* L.).

According to the EU regulations [10], stability studies are necessary for the market authorization of an herbal medicinal product. This is another part of the quality requirements where HPTLC has a place. Unless justified, the accepted variation in content of constituents with known therapeutic activity during the proposed shelf-life is ± 5% of the declared assay value. Whereas when constituents with known therapeutic activity are unknown (markers are used in this case) the accepted variation is ±10% of the initial assay value.

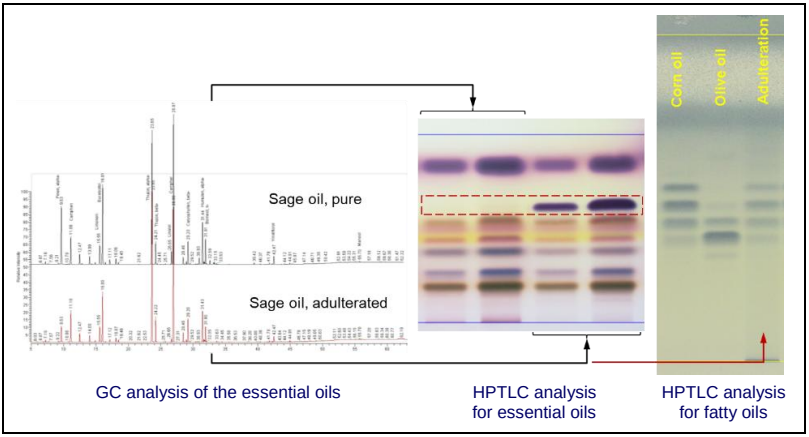


Figure 4. Detection of adulteration of sage essential oil with a fatty oil by HPTLC. The adulteration is not detected by the analysis of the essential oil by gas chromatography (GC), but by HPTLC, where the triglycerides of the fatty oil produce a distinctive zone in the chromatogram of the adulterated oil (see red rectangle). The HPTLC analysis of the essential oil using a method for fatty oils shows that the fatty oil used for the adulteration produces a profile similar to that of corn oil.

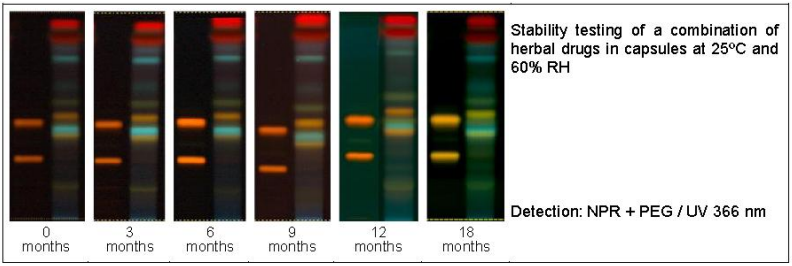


Figure 5. HPTLC chromatograms used for a stability study of a combination of herbal drugs in capsules. For each time point, left track corresponds to reference solution and right track to test solution.

Nevertheless, since the herbal drug or herbal preparation in its entirety is regarded as the active substance, the determination of the stability of the constituents with known therapeutic activity or the markers is not considered sufficient. The stability of other constituents of the herbal drug or the herbal preparation should, as far as possible, also be demonstrated. The HPTLC fingerprint chromatograms are appropriate in this case, as it is shown in Fig. 5.

3. Recent improvements in the Pharmacopoeias

TLC has been included since decades in most of the Pharmacopoeias as identification tool for herbal drugs and herbal preparations. However, a number of issues have been observed over the years with this traditional use, in part due to the technique and in part due to the nature of the herbal materials. First, the variability of the chromatograms obtained, partially due to the inherent variability of the herbal drugs (differences between batches), but also to the lack of intra- and inter-laboratory reproducibility of the analysis. Second, the difficulties on the description and interpretation of the chromatograms. Over the years, the evolution of the description systems in the Ph. Eur. has improved its accuracy by changing from plain text to the use of tables. However, it remains a challenge to describe the natural variability of an herbal drug in a single description of the TLC/HPTLC fingerprint, including the selection of zones to be considered/described and the definition of their position, color and intensity in a way that avoids, as far as possible, ambiguity in the interpretation. At the end, the qualified person in a laboratory has to decide whether the fingerprint of a sample is compliant with that described in the pharmacopoeia. Moreover, in most of the cases,

the pharmacopoeias do not include any system suitability test for qualifying the plate before any interpretation of the chromatograms [11,12].

In order to solve these issues, improvements have been implemented in several pharmacopoeias, that published new general chapters on the analysis of herbal drugs and preparations by HPTLC: the general chapter 2.8.25 (*High-performance thin-layer chromatography of herbal drugs and herbal drug preparations*) in the 9th edition of the European Pharmacopoeia [1] and the general chapters <203> (*High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin*) and <1064> (*Identification of Articles of botanical origin by High-Performance Thin-layer Chromatography procedure*) in the USP 38 – NF 33 [13].

The first step for **improving reproducibility** is the use of HPTLC instead TLC, because inter-laboratory trials performed in the framework of the Ph. Eur. have demonstrated the higher reproducibility of this technique [11,12].

The second step is the standardisation of the methodology. This is the use of standard operating procedures (SOP) that include detailed descriptions of all relevant parameters, such as preparation of the sample, plate setup and handling, sample application (as band), chamber geometry and saturation, humidity control, developing distance, derivatisation procedure and observation, documentation (as electronic images), and evaluation of the chromatograms. The standards used nowadays in the Ph. Eur. and the USP are following the recommendations of the HPTLC Association (www.hptlc-association.org). The new general chapters of the Ph. Eur. and the USP include detailed description of the common part of the HPLC methodology, while the specific information regarding each method is included in the corresponding individual monograph.

The qualification of the plate is a key factor for improving reproducibility of the HPTLC analysis. It answers the question: Was the HPTLC analysis properly done? Only qualified plates should be used for evaluation of results and for comparison of results of different analyses of the same herbal drug or preparation, performed in the same or in different laboratories.

There are different approaches to the qualification of the plate. In the USP, it is based on two or more reference substances that have just separable R_F or (in most cases) a reference extract, for which results should match description of colors and position, within a specified range. In the HPTLC Association, it is mainly based on reproducible and standardized

HPTLC results, with the definition of exact R_F values. Finally, in the Ph. Eur. a system suitability test (SST) is described in each method and it is based on the separation of 2 substances that have similar retardation factors (R_F values) but that are barely separable under the specified chromatographic conditions. An example of selection of a pair of substances for SST is shown in Fig. 6 for an analysis of flavonoids and phenolic acids [11]. The SST is normally applied on the first track and, in addition to the qualification of the plate, it is also used for normalization of the electronic image of the plate.

As mentioned earlier, one of the main issues with the traditional use of TLC was the **description and interpretation of the chromatograms.** The description has to take into account the sequence and characteristics of the zones (number, position, colour and intensity). The interpretation of the intensity was highly subjective, because there was not a reference to compare with. In order to improve this situation, the new chapter 2.8.25 of the Ph. Eur. has introduced the use of **intensity marker**. The intensity marker is selected from the reference substances used. The reference solution (containing the intensity marker) is applied on adjacent tracks at the normal concentration and diluted one to four. The intensity marker will give different intensities in the two tracks. The intensities of the zones of the chromatogram of the test solution are described comparing to these two different intensities. The meaning of the descriptors used in the Ph. Eur. is illustrated in the Fig. 7.

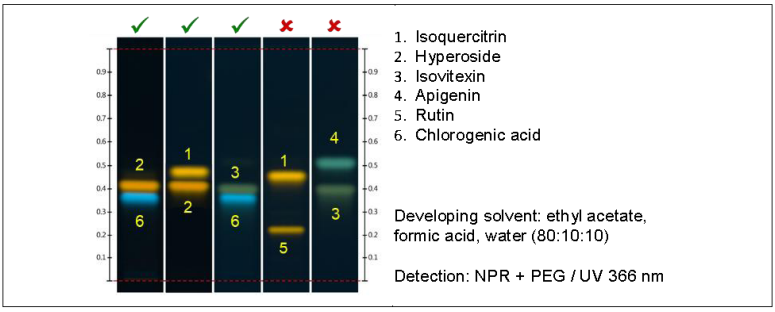


Figure 6. Selection of a pair of substances for the system suitability test (SST) in the analysis of flavonoids with the developing solvent ethyl acetate, formic acid, water (80:10:10 V/V/V/V) on a HPTLC silicagel 60 F_{254} plate. The pairs of the first two tracks are suitable for the SST, and preferable to the pair of compounds of the track three, which are very close together. The pairs of the two last tracks are not suitable because the two compounds tested are too far from each other. NPR: Natural product reagent, PEG: Polyethylenglycol 400.

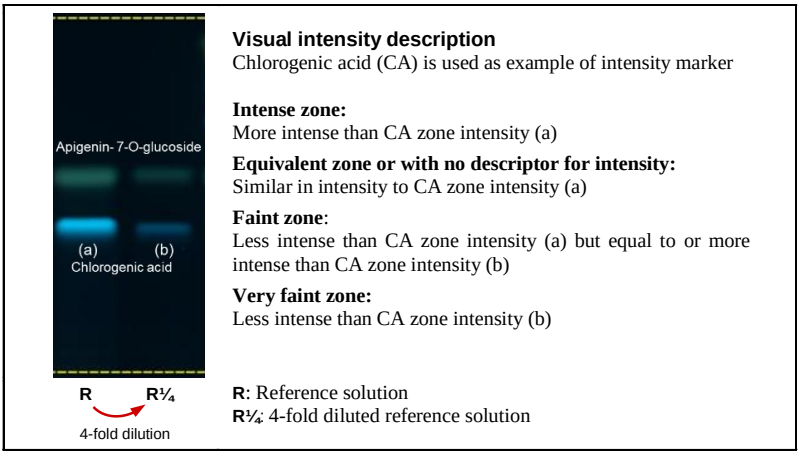


Figure 7. Visual intensity description according to the general chapter 2.8.25 of the Ph. Eur., using chlorogenic acid as example of intensity marker.

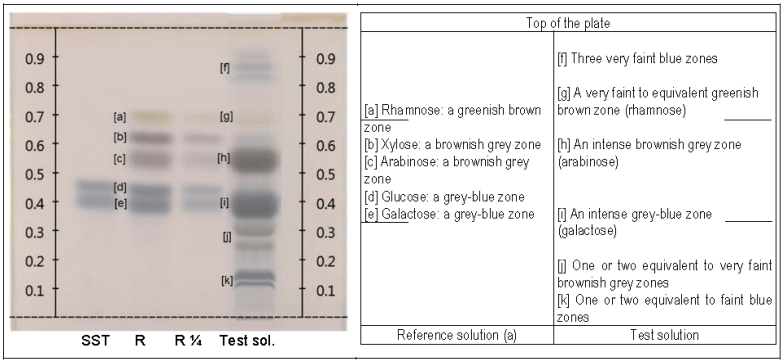


Figure 8. Proposal of description for the identification of acacia and acacia spray/roller dried by HPTLC using the method developed as described in the present chapter (intensity marker: galactose). R: reference solution (a), R¼: 4-fold diluted reference solution (a).

Together with the implementation of the general chapter 2.8.25, the 9th edition of the Ph. Eur. has introduced another significant improvement for the interpretation of the chromatograms, the publication of **colour pictures of the chromatograms**. They are not published in the Pharmacopeia itself but in the Knowledge database. They are not mandatory, but given only as

information. Pictures include type chromatograms, helping with the identification of the zones referred to in the description table of the chromatograms of the individual monographs, as well as pictures of several batches of the herbal drug or preparation to show natural variability. Figure 8 shows an example of a type chromatogram and proposal of description of results according to the new chapter 2.8.25 of the Ph. Eur. for acacia and acacia spray/roller dried. Other Pharmacopoeias and Compendia [13-19] also started to publish pictures of the chromatograms.

4. Development of an HPTLC method

The routine chromatographic identification of polysaccharide rich herbal drugs and preparations thereof, such as gums, mucilages and starches is based on the TLC analysis of the monosaccharides released by hydrolysis. In the 8th edition of the Ph. Eu. [20], TLC identification was described in a limited number of the monographs related to this class of compounds and HPTLC was not described in any of them. In addition, the sample preparation was tedious and applied different conditions of hydrolysis. The TLC methods had also different mobile phases and three different detection reagents. With the aim of setting up an improved and harmonised general method for identification of this type of herbal drugs and preparations, suitable for routine quality control, an HPTLC method was developed and adapted to the requirements of the new Ph. Eur. chapter 2.8.25, published in the 9th edition [1].

In the next paragraphs, the parameters studied and the conclusions obtained will be summarized [21].

For the **preparation of the test solution**, the hydrolysis at 100 ºC (1h, oven) and 120 ºC (1 h, oven or autoclave), using different concentrations of trifluoroacetic acid (TFA) (100 g/L and 230 g/L) were tested. After the hydrolysis, the need of evaporating the acid (which is time consuming) was tested, as well as the suitability of the use of methanol instead of water as final solvent. TFA at 100 g/L, 120 ºC, during 1 h, were the conditions selected for hydrolysis, and the subsequent treatment was considerably shortened by avoiding evaporation of the acid. Methanol as final solvent was considered suitable.

The starting point for the **chromatographic separation** were the TLC methods already described in the Ph. Eur. [20] for ispaghula (seed and husk) guar and guar galactomannan. The separation was performed using one development with acetonitrile, water (85:15 v/v), in a saturated chamber. The main problem of this system was the relatively low R_Fs and a limited separation of the monosaccharides. With the aim of improving the separation, other proportions of this binary solvent system were tested, as well as the addition of different proportions of a third solvent, either

acetone or 1-propanol. Saturation with different solvents as well as the use of an unsaturated chamber were also investigated. And, finally, the suitability of multiple developments was verified. An optimised separation was obtained using HPTLC silica gel 60 F_{254} plates conditioned to a relative humidity of 33%, and double development with acetonitrile, water (85:15 v/v) in an unsaturated chamber.

For the **detection**, four derivatisation reagents were tested, and diphenylamine - aniline - phosphoric acid reagent was selected, with observation under white light.

Three pairs of reference substances were tested as candidates for the **system suitability test (SST)**. Galactose and glucose were considered the most suitable pair as SST for the qualification of the plate.

Finally, as **intensity marker**, a monosaccharide was individually chosen for each herbal drug or herbal preparation.

A description to which the chromatogram has to comply was prepared for each herbal drug/preparation, together with the type chromatogram, according to the general chapter 2.8.25 of the Ph. Eur. A proposal for acacia and acacia spray/roller dried is shown in Fig. 8. The application of the method to different herbal drugs and preparations is shown in Fig. 9.

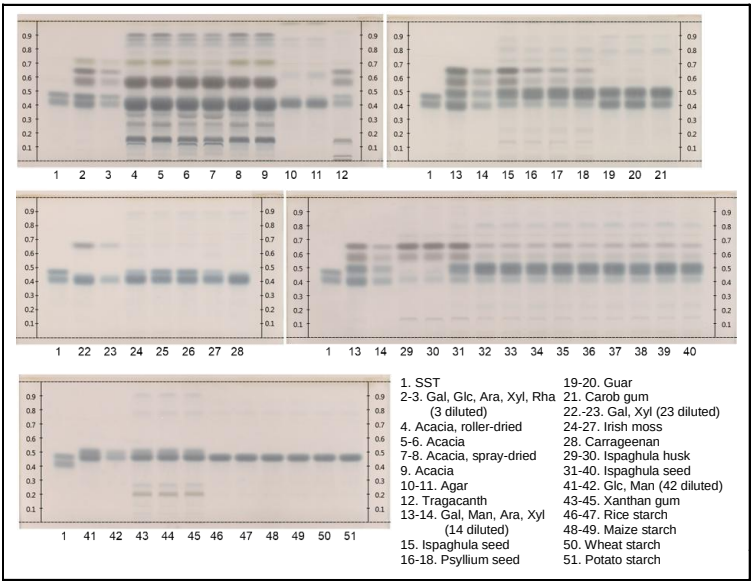


Figure 9. HPTLC chromatograms of several polysaccharide containing herbal drugs and preparations, using the method developed as described in the present chapter.

5. Comprehensive HPTLC fingerprinting

HPTLC is nowadays adopted by the major pharmacopoeias of the world for analysis of herbal drugs and preparations. The current use is generally limited to the visual observation of the fingerprints for identification and detection of adulterations and falsifications. However, standardisation of methodology offers the necessary reproducibility that allows a deeper exploitation of this technique in the field of herbal products. Indeed, the HPTLC fingerprint, as an electronic image of the chromatogram, when generated by a standardized methodology under use of suitable instruments and software, and qualified by a SST, can also provide quantitative information based on the intensity of zones [22]. This information can be used for performing limit tests (e.g. for adulterants), determining content of active constituents or markers, or development of other quality parameters that need quantitative data. This quantitative information can be accessed by converting HPTLC fingerprints (electronic images) into peak profiles from images (PPI) by calculating the luminance from the average of RGB pixels of each line of the track and then plotting it against the RF values. Figure 10 shows this process. The height of the peak in luminance is used as measure of intensity and is compared with the corresponding peak of reference material. This comparison is far more objective than the simple visual comparison of zones intensities.

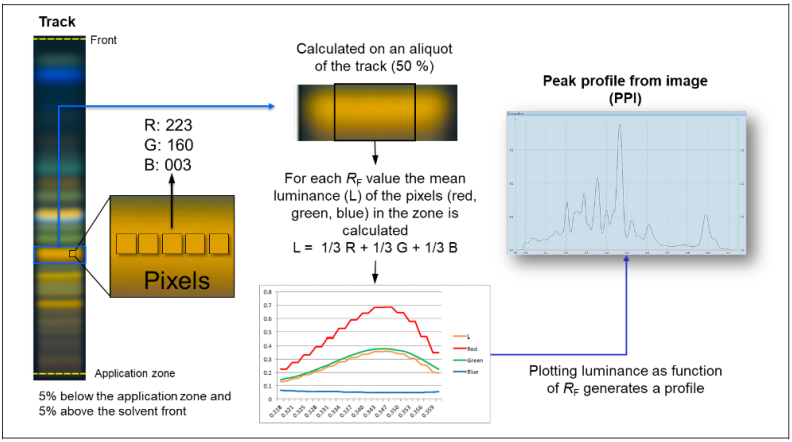


Figure 10. Transformation of the electronic image of the HPTLC chromatogram into the corresponding peak profile from image (PPI).

Based on that, the concept of "comprehensive HPTLC fingerprinting" has been developed [7]: HPTLC fingerprints used for identification, are converted into PPI and used for obtaining information on purity and/or strength/content of the herbal drug or preparation analysed. A single analysis by HPTLC gives comprehensive information on quality and allows the simplification of the quality control process.

The proof of concept has been illustrated in the case of the quality control of the root of Korean angelica (*Angelica gigas* Nakai), known as dang gui and commonly used in the Republic of Korea [7]. It contains decursin and decursinol angelate, which are used as quality markers and a minimum content is required. Two other species of *Angelica*, Japanese angelica (*A. acultiloba* (Siebold & Zucc.) Kitag) and Chinese angelica (*A. sinensis* (Oliv.) Diels), also known as dang gui in their respective countries of origin, can be considered adulterants when mixed with the former. However, the roots of these two species contain *Z*-ligustilide, which is not present in the root of *A. gigas*. Comprehensive HPTLC fingerprinting of this herbal drug, means than after a single analysis, it is possible to retrieve the following information:

- Identity, based on the visual observation of the fingerprint.
- Distinction from the roots of other 27 *Angelica* taxa and related Apiaceae species, also based on the visual observation of the fingerprint.
- Discrimination of roots of *A. gigas* adulterated with the roots of *A. sinensis* or *A. acutiloba*, based on the PPI analysis of the presence of *Z*-ligustilide. A 5% of adulterant or even less can be clearly detected.
- Compliance to an established minimum content of the sum of decursin plus decursinol angelate, based on the PPI analysis of the peak that is compared with the corresponding peak of the reference material.

Another example in which the use of comprehensive HPTLC fingerprinting can reduce the number of analyses needed for quality control is the refined dry extract of ginkgo leaf (*Ginkgo biloba* L.) [23]. The USP monograph [13] prescribes an HPTLC identification looking at the fingerprint of flavonoids, and an HPLC assay of flavonoids performed on the aglycons (quercetin, kaempferol and isorhamnetin) released after hydrolysis. The flavonoid content required is 22-27%. The extract can be adulterated with pure flavonoids (e.g. quercetin and rutin) or other herbal extracts rich in flavonoid glycosides releasing quercetin by hydrolysis. The objective of the adulteration is to give the same flavonoid content using less ginkgo leaf extract. For this reason, the USP includes an HPLC limit test for rutin and

quercetin. Comprehensive HPTLC fingerprinting applied to ginkgo products is able to check, after a single analysis:

- Identity of the extract contained in the product, by visual evaluation of the fingerprint.
- Adulterations with quercetin and/or rutin rich materials and the nature of the adulterant, also by visual evaluation. Most common adulterants can be detected: the extracts of sophora fruit or flower bud (*Styphnolobium japonicum* (L.) Schott, syn: *Sophora japonicum* L.), and of buckwheat herb (*Fagopyrum sp* Moench), as well as pure rutin or quercetin.
- Compliance to the USP limit test for quercetin and rutin, using the PPIs obtained from the fingerprints acquired from different detections on the same plate. Results from the HPTLC analysis show a very good correlation with those obtained by HPLC according to the USP. Consequently, the HPLC limit test prescribed by the USP could be avoided by means of a deeper exploitation of the fingerprints obtained in the HPTLC analysis for identification.

6. Conclusions and future prospects

HPTLC is a simple, visual and pragmatic technique, capable of delivering reliable and reproducible results, based on standardized methodology and the use of SST for plate qualification. The improvements introduced in the Pharmacopoeias, especially in the Ph. Eur. and the USP, together with the instrumentation and software allowing to record normalized electronic images of the chromatograms (fingerprints) and their transformation into peak profiles (PPI), open the door to retrieve quantitative information from the analysis performed for identification. Based on that, the concept of comprehensive HPTLC fingerprinting has been introduced. It allows simplification of quality control processes for herbal drug/preparations/products, because from a single HPTLC analysis, information on identity, purity and content can be obtained.

Additionally, HPTLC results generated on different plates can now be compared based on the electronic images of the fingerprint. Images can be stored in an electronic atlas or even in a cloud, which can be accessed by different labs, enabling global exchange and collaboration.

Herbal drugs and herbal preparations have a high chemical complexity and they are considered the active pharmaceutical ingredients in their entirety [10]. The traditional approach of assaying a selected marker, that often represents only 0.02 to 5% of the total composition, has a limited significance for the level of quality and it is under discussion. A more

holistic approach, considering a wider range of constituents would be suitable. In this context, the HPTLC chromatographic profiling of herbal drugs/preparations/products appears as an essential tool to stablish its quality [24,25]. Probably, chemometrics will help on that, but how it will develop is still unknown, because it is not only depending on the technical capacities, but on how to establish official criteria of acceptance that can be implemented in a Pharmacopoeia.

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