PANEL DISCUSSION REPORT

HPTLC International Symposium of BASEL
Date: 2011, July 7th

Chair: Prof. Matthias HAMBURGER, Switzerland.

Invited Speakers	
Prof. Zheng-Tao WANG	Shanghai University of Traditional Chinese Medicine, China
Dr. Clemens ERDELMEIER	Dr. Willmar Schwabe Pharmaceuticals, Germany
Dr. Eike REICH	Camag Laboratory, Switzerland
Dr. Gudrun ABEL	Bionorica SE, Germany
Dr. Werner KNÖSS	Federal Institute for Drugs and Medical Devices (BfArM), Germany
Dr. Troy SMILLIE	University of Mississippi, USA
Dr. Bernd RENGER	Bernd Renger Consulting, Germany

CONTEXT: HPTLC applications/ Herbal drugs & medicines

Prof. Hamburger explains the aims of the discussion. Herbal drugs and herbal medicines are among the most important areas where planar chromatography is being used as an analytical tool. To address some of the associated issues experts with various backgrounds were invited to discuss this topic. Scientists from regulatory bodies, industry, and academia from several continents with a strong background related to the quality insurance of herbal drugs gave their opinion.

QUESTIONS / TOPICS

The pharmacopoeias are among the most important documents for quality control of herbal products, and most monographs for herbal drugs include TLC methods for identification.

Prof. Hamburger's first set of questions is addressed to the industry, and to those developing official monographs: Are the monographs really applied in practice, or does industry rather use more suitable in "house" methods? How are these TLC methods being developed? How much interaction is there between manufacturers, equipment suppliers, and academic groups?

According to Dr. Abel, TLC methods found in the pharmacopoeias are used in part; they may have to be modified depending on the finished product, but they are always an important part of the specification.

Dr. Erdelmeier agrees. If there is a monograph for a raw material or for an extract in a pharmacopoeia, then he uses it.

Dr. Reich points out that requests for monographs are usually made through the national authorities to the responsible expert groups of the pharmacopoeia which develop methods accordingly. A draft is published e.g. in Pharmeuropa for a period of 90 days during which other labs can evaluate it and give their comments. Any problem will be addressed in further discussions, and the monographs are modified as necessary before adoption by the Pharmacopoeia Commission.

Prof. Hamburger: Is the process driven by the Pharmacopoeia Commission or by industry?

Prof. Wang replies that in China, TLC methods are a very popular part of monographs. They are important for identification of herbs, and helpful for starting materials and finished products. He reminds the audience that in the morning, he talked about an atlas of monographs that includes herbal materials, extracts, and finished products. There is some conflict between academic research and regulation because advanced instrumentation allows a beautiful fingerprint. But in China it is up to the Drug Control Institute to accept or reject a product. The chromatographic resolution of a method is often an issue.
Fingerprints may differ for plants from different localities. Sometimes 10 bands are required to identify a plant clearly, sometimes 12 or 8, and the government or the Pharmacopoeia and Drug Institute base the result on similarity

Dr. Abel gives a comment on the cooperation between industry and the Pharmacopoeia Commission saying that it depends on the person that proposes the monograph. The Pharmacopoeia Commission wants a herbal reference standard at the time of the publication in the pharmacopeia. If the description is not detailed enough to check whether the samples meet the requirement, the time window of 90 days for commenting on a draft monograph is not enough. The biggest problem for the Pharmacopoeia Commission is the availability of certified reference standards.

Prof. Hamburger: How are the TLC methods being developed in the USA?

Dr. Smillie specifies that in the research center at University of Mississippi they are utilizing the entire spectrum of methods in their work with botanical products, ranging from microscopy to TLC and HPLC. They can provide standards and reference material as well as chemicals, but they have not contributed very much to any of the USP monographs or others.

Dr. Knöss: From the regulatory point of view, the difference between in “house” or Pharmacopoeia monographs is absolutely clear. In pharmacopoeia monographs, methods are validated. Companies don’t need to provide any more data. With “in-house” methods they have to do an entire validation. Depending on the company size, this may be a major effort.

At the moment, TLC descriptions in pharmacopoeia monographs are very schematic. What is the future? Will it be pictorial? Are there other ways of dealing with this for the future?

Dr. Reich clarifies that he is not representing the European Pharmacopoeia in this panel. He personally thinks that there are things that need to be changed, in particular, that HPTLC will sooner or later be documented and distributed through images. He refers to the example of black cohosh. It is the first time that the European Pharmacopoeia uses images to illustrate a result for HPTLC. This is a more efficient way to store and distribute information.

Dr. Renger adds a further comment to the last point of the discussion: Pharmacopoeia methods are considered validated, but he finds that their use is increasingly questioned by inspectors. He proposes a qualification that confirms the ability to perform a pharmacopeia method. This is something that adds a new layer to the pharmaceutical industry.

Dr. Knöss specifies that currently it is really a point for the inspection, and not for the application. Validation refers to the methods, which, of course, can be carried out in a unsuitable way. The validation has to show the suitability of the system and its capability to deal with the analytical question to be addressed.

Dr. Reich explains that HPTLC methods that CAMAG Laboratory recently contributed to the pharmacopoeia have suitability test build in to the method, although they are not explicitly described as such. And if the lab can meet those tests, the data is qualified. This could be a standard practice across the industry. If, for example, someone wants to compare data from a stability study over a certain period of time,

one has to make sure that the investigated substances are about at the same position, whether people call it system suitability test or not. They are qualifying their data, and the same could be done with the fingerprint or with the description of the chromatogram, but not in a table form as is the current practice of the European Pharmacopoeia. The USP is also stepping up into this direction by introducing suitability tests on the plate. The USP Dietary Supplements Compendium is being revised. The second edition will be published at the end of this year and will include such tests.

Prof. Hamburger: The use of reference materials is often complicated by the fact that standards of an appropriate quality can be very expensive or not available at all. Is the general tendency going towards certified herbal drug standards as a reference, and what is the future with these monographs?

Prof. Wang: In the Chinese Pharmacopoeia for TLC identification, they use two kinds of references material; herbal reference drugs and reference compounds, sometimes one but often both. What about Europe?

Dr. Reich: Tendency has been in the European Pharmacopoeia to use easily available reference substances as markers for the description. More recently, herbal reference standards have been introduced for some monographs. These are powered material or extracts that are to be used as standards.

Prof. Wang confirms that they do the same in China. In China scientists are now trained to use herbal reference material, because active chemical reference material is always in short supply. Some of the purified compounds are very rare due to stability problems. Herbal material is also a problem. It must be decided which one to use, how to select it for each herbal product, and how to accommodate varieties from different localities which result in differences in the TLC fingerprints. So now, they hope to use the standardized extracts as a reference material to qualify raw material.

Prof. Hamburger: TLC is used mainly for fingerprint analysis. Fingerprints are visually highly attractive and also quite diagnostic, but the visual assessment is subjective.
Is there a need for a more objective way of assessing similarity of fingerprints, and what would these methods be?

Dr. Smillie thinks it could be very interesting and very useful to build up a database of HPTLC profiles of herbal drugs. Very often, one doesn't know whether differences in the fingerprint of a sample and a standard reference plant material are due to seasonal variability, locality, and other environmental factors. Thus, building a library of a wide collection of authenticated materials would be paramount.

Dr. Erdelmeier's view is that the current way is sufficient for their work, but he specifies that he only uses TLC as a qualitative tool in his research. They stay in the area where they need more an answer like yes or no. For him, this is sufficient for what they can do now with the equipment available. However, he agrees he would need some more advanced methods for assessment if he would use TLC for more sophisticated research questions.

Dr. Renger: Even for non-quantitative but qualitative applications, he would consider chemometrics as the way out of the trap of not having really specified, really identified, and qualified reference materials. Using chemometrics and a certain set of samples to make decisions would be a way out for him.

Dr. Abel agrees with the last proposition. She thinks the important thing is the standards for matching because the variability from herbal drug to herbal drug is different. It is important to avoid in the end that only 10% of herbal drugs would meet the requirements. So putting in place this representative set of samples for the qualification is the decisive task.

Dr. Renger replies that it is absolutely right but he would fear the use of extracts as reference materials because those that would supply the reference materials would in some way or the other influence what is considered to be the correct herb.

Dr. Reich thinks that they are touching here the very basis of the matter. People are trying to classify herbal material. That's not going to happen. Each plant has its own fingerprint. So according to him, one should step back a little bit and use the concept of FUZZINESS that was introduced from China. Actually Professor Xie Peishan has published a lot on this; chemometrics can be used to access that.
Coming back to the previous question, concerning reference materials; he thinks there is no such thing as a single reference herb. Rather, there should be a series of reference herbs. He envisions a database where different researchers from different countries can upload their own information, and the more information about a specific herbal drug uploaded, the better our understanding of the variability of that herb.
Unlike the pharmaceutical industry, which has a rather good control over their raw materials, the botanical and dietary supplement industry does not have a comparable control over their herbal raw materials. There is a global market of herbal raw materials, intermediate and finished products being shipped and exchanged. Every time these goods change hands, one has to do some identification. Now what will it be at the end? The future is to collaborate extensively at an international level using a standardized methodology. This is what he has been proposing for the past 7 years. If a standardized methodology is used, samples can be analyzed with the same method, and all data can be compared. There has to be some way of demonstrating that the data is valid, e g. using a system suitability test before computing results to find whether the samples matches the desired cluster. The other option would be of course to have a reference extract that is produced from multiple batches as a pooled sample, but nobody will be happy with that.

Dr. Knöss would like, at least for now, to distinguish between looking into the future for the scientific part and for the regulatory part, because the methods that are being discussed at the moment are under development. Looking at this mixture of techniques and databases appearing to be derived from chemometrics could get us into trouble if it becomes a regulatory requirement because it will just demonstrate that herbal medicinal products are not that reproducible in quality, as believed for some time. He feels one has now to look at what the outcome of this system is. The biology of metabolomics and related techniques will provide a better explanation, maybe in 5 or 10 years, about the usage of medicinal plants and these multi-components mixtures. People have to look for ways to deal with the variability since the regulator wants to know which of these extracts is the one that represents the

best quality, the best safety profile, and the higher efficiency. At the moment we are not that far along the way. Therefore he would be careful just to transfer these new methods into the regulatory requirements.

Prof. Hamburger corrects that there was maybe a misunderstanding. He emphasized that he doesn't want to bring that at a regulatory level but he implies the need to gather data to make a uniform decision on whether such data can be used in the future. This is really a bit more looking into the future somehow.

Dr. Knöss doesn't object to this clarification. They are scientists; they have to follow this path.

Prof. Hamburger: Can HPTLC be used for quantitative analysis of herbal extracts. What is the potential?

Prof. Wang: In the Chinese Pharmacopoeia, the use of scanning densitometry as a quantitative method has been reduced greatly: Currently, there are only 15 monographs using quantitative TLC and 2 years ago, they finished a draft. The younger members of the pharmacopoeia committee hope to eliminate all the quantitative TLC, but he and a few of his colleagues still argue against it.

Dr. Reich agrees with Dr. Erdelmeier's previous statement. He adds that he would not want to change a good quantitative assay. However, he thinks there is a potential when new monographs are developed. He agrees with Dr. Abel when she says there are some methods that are slow and they are slow so as to obtain a perfect separation. Some HPLC methods are slow as well because they have to clean up the column. HPTLC doesn't really have that problem and it's possible to obtain a quantitative assay in a much shorter time without the separation of all the compounds when looking for just certain markers.
Otherwise, he thinks if people have a well-developed fingerprint, they could also take that fingerprint and add the reference substances in different volumes and thus do a quantitative assay. At least a semi-quantitative assay is possible to qualify their material. Anyone trying to pass an assay by spiking the material can be uncovered because the fingerprint will not be of the same intensity. He thinks there are some trials being done, particularly at the University of Regensburg, where they are developing methods that are going to be proposed to the European Pharmacopoeia. HPLC and HPTLC assays are evaluated in parallel to demonstrate that the performance characteristics of these two methods are equivalent in many cases.
So it would be a good option for people who want to employ HPTLC to let them do that, provided the performance is sufficient. But what can be done with people who do not want?
It is difficult to force someone to abandon HPLC and buy another system. But he thinks there are points to be made in using HPTLC as a quantitative tool if it is done properly and validated properly.

Prof. Hamburger: what are the specific issues with HPTLC validation?

Dr. Renger: The specific issues in TLC are not different from those in HPLC. One only has to consider that one is dealing with herbals, and that the variability of

biological materials is far higher than with synthetic molecules. He agrees with Eike Reich. If there is a very good fingerprint that distinguishes between the different components the most difficult step towards a quantitative procedure is done. With a very good fingerprint, it's possible to develop a quantitative method. But one cannot use the requirement of ICH Q2R for something which is a biological and natural material. He refers here to another example of a biological material, blood products. Plasma comes from donators who are very different, in different seasons, different countries.

Dr. Erdelmeier doesn't have an answer to the validation question. He thinks that the discussion about advantages and disadvantages of quantitative analysis by TLC or HPLC has been going on for quite a while. He thinks that the choice is often more an issue of company philosophy. In some companies, some people stick more to HPLC, and may be in another company, people prefer HPTLC. That can't be changed.

Prof. Hamburger: From the regulatory side, how often do you see quantitative HPTLC assays in documents that are filed for herbal products?

Dr. Knöss: The German Agency rarely receives application files with a quantitative HPTLC assay. Like Dr. Erdelmeier, he thinks that HPLC was the method used for quantitative assays in the past decades. Thus, these are well established methods in many companies. They use HPLC methods for many of the common herbal drugs.
He draws the attention of the audience to one aspect highlighted by Dr. Renger's presentation: Variability and variance is typically a little bit higher than with other methods such as HPLC. One can deal with that but must also face it. He feels that the chemometric analysis of fingerprints could possibly give much more information than just an assay of a single compound. However, he doesn't want to be misunderstood. Fingerprints are part of the current requirements. Maybe in 5 to 10 years, the fingerprint is of higher importance than a quantitative assay for a single substance in a multicomponent mixture such as a plant extract.

Prof. Hamburger: Where do you see HPTLC in the herbal area in 5 to 10 years? Where are the areas for development? What are the most important needs for improvement?

Dr. Smillie strongly believes in building a database to help start these reference standards. Until people start agreeing on what is a proper method, how it is developed, how it can be validated, people will all use their different methods and will not come to the same conclusion. Thus, some standardization is required.

Dr. Renger thinks that the available equipment does not need further improvements. He guesses that the Achilles' heel, the main source of problems today is in the sample preparation and in the plates. Thus, improvements are needed in these areas.

Prof. Hamburger: So you say the hardware is good, how about the plate? Is there room for improvement? New concepts of generating the stationary phase in planar chromatography, is this the future?

Dr. Renger: There is always a place for certain exotic and new developments, but if people take a look to the data they will find that they may gain 0.1% - 0.05% percent

in precision by doing something in that direction. But they will gain 2% if they improve upstream, e.g. in the sample preparation. This is not limited to herbals, it's applicable to all analyses, and this aspect has not been considered enough up until now.

Dr. Abel thinks people should use TLC in areas where the method has clear advantages over other analytical methods. One should widen the spectrum of application but not try to compete with other methods where no obvious advantage can be seen.

Dr. Reich would actually join Troy Smillie in what he has said. He thinks a standardized approach to HPTLC for plant identification is a goal that scientists should set for themselves. Collaborating for the next 5 years could lead to a shared database of methods and chromatographic images to which everybody could contribute and which everybody could use. As far as chromatographic plates are concerned, he sees a real limitation in terms of their quality. He wishes that they were of comparable quality to those of 10 to15 years ago, where quality was much higher.

Prof. Hamburger thanks the panel participants and concludes the round table discussion.