imaging process must be completely controllable, predictable, and reproducible to ensure that differences between two images are caused only by differences in the sample.

Because stability tests are mainly performed on herbal medicinal products, all analytical work has to comply with GMP regulations.

C. Quantification of Marker Compounds

1. General Aspects

Simplified sample preparation, the option of specifically optimizing chromatography for the separation of selected compounds, and the possibility of specific postchromatographic derivatization are advantages of quantitative planar chromatography. Because of the single use of the HPTLC plate, contamination of the system is not an issue as it is for sequential techniques like column chromatography. Nevertheless, most fingerprint methods that are used for identification are not suitable for quantification without adaptation. The principal restriction is that of separation power. If the sample contains too many components, a useful fingerprint may still be obtained but baseline separation of all substances as a requirement for quantification may be difficult to achieve. However, aside from resorting to gradient techniques such as AMD, the best solution is the optimization of the method for the separation or quantification of selected marker compounds. The possibility of successful quantitative HPTLC of herbal medicinal products is documented by the fact that in the last two years over 90 papers were published on this subject. The majority of these publications concern quality assurance of traditional Chinese medicines. The particular advantages of quantitative HPTLC as a complementary technique to HPLC and GC are discussed by Xie (40).

Quantification is usually performed by scanning densitometry. Scanning each track of the plate with a light beam of selectable dimension and wavelength, the absorption or fluorescence of the sample components is measured. The raw data in the chromatogram are integrated and compared to those obtained from a set of known standards chromatographed on the same plate.

2. Requirements

Quantification of marker compounds requires suitable instrumentation (see Chapter 5 of this Handbook) to perform each step of the HPTLC analysis accurately and precisely. All steps of the HPTLC method should be optimized and standardized to ensure reproducible data. It is necessary to validate quantitative methods according to ICH guidelines Q2A and Q2B (12,13). If possible, quantitative evaluation should be performed prior to derivatization unless the target compound must be detected by a chemical reaction.

3. Example

Pharmacopoeial methods described for the fingerprint identification of bearberry (*Arctostaphylos uva ursi*) typically result in a chromatogram similar to that seen in Fig. 14a when viewed under 254 nm UV light. An important quality-related question aside from the identity of a bearberry preparation or finished product is its hydroquinone content. In the chromatogram hydroquinone is seen as a very faint zone close to the solvent front. The more intense zone just below is that of gallic acid. Figure 14b shows the chromatogram of different amounts of gallic acid [migration distance (MD) = 45 mm] and hydroquinone (MD = 50 mm) after densitometry at 287 nm. There is a secondary solvent front at MD = 52 mm that interferes with the quantification of hydroquinone. Optimization of the chromatographic conditions (Fig. 14c) results in complete separation of the hydroquinone peak (MD = 57 mm) from the solvent front (MD = 70 mm) and allows quantification of hydroquinone in bearberry preparations (tracks 5–10).

D. Method Development

There are many new herbal drugs for which no TLC-related information can be found in the literature. In other cases the official methods or other established procedures are not adequate to answer a given analytical question and the usual optimization attempts (HPTLC instead of TLC plates, improved sample application, changing chamber conditions, chemical derivatization, multiple detection, etc.) have failed. At this time it becomes necessary to develop a new HPTLC