SPECIAL GUEST EDITOR SECTION

Validation and Quality Assurance of Planar Chromatographic Procedures in Pharmaceutical Analysis

KATALIN FERENCZI-FODOR, ZOLTÁN VÉGH, and ANIKÓ NAGY-TURÁK
Chemical Works of Gedeon Richter Ltd., H-1475, Budapest, Hungary
BERND RENGER[1]
Byk Gulden Werk Singen, Robert-Bosch-Straβe 8, D-78224 Singen, Germany
MARCO ZELLER
Solvias AG, CH-4002 Basle, Switzerland

Within the process of the International Conference on Harmonization (ICH), 2 guidelines were released containing a standardized terminology, a verified model of requirements for the validation of analytical procedures, and some guidance in the practical aspects of conducting validation studies in pharmaceutical analysis. For planar chromatographic procedures, which may be used at different levels either in qualitative identity testing, assays, semiquantitative limit tests, or quantitative determination of impurities, this paper tries to transfer these formal requirements into practical approaches for validation. Basic acceptance criteria for evaluation of validation experiments based on practical experience are proposed. In addition, selected parameters for robustness testing of given procedures and quality assurance of quantitative planar chromatographic testing by control charts is described.

The success of high-performance liquid chromatography (HPLC) over the past 15 years has tended to push planar chromatographic techniques (thin-layer chromatography [TLC], high-performance, thin-layer chromatography [HPTLC], and over-pressured layer chromatography [OPLC]) somewhat into the background. However, planar chromatography remains one of the obligatory identification tests for pharmaceutical ingredients. In its semiquantitative mode, based on visual evaluation, it is still predominant as a limit test for impurities, especially if they are not or only poorly detectable by HPLC (1, 2). In addition, the progress in sorbent layers and instrumentation has led to an improvement of the reliability of quantitative planar chromatography, making this technique an economical alternative that competes with and complements HPLC (3, 4). Quantitative planar chromatography using scanning densitometry was included in the latest revision of the General Chapter on TLC of the European Pharmacopoeia and acceptance criteria for resolution and limit of detection are given (5).

The suitability of any analytical procedure for its intended use in pharmaceutical analysis must be based on objective validation data. A set of 2 guidelines on the validation of analytical procedures was prepared and adopted under the auspices of the International Conference on Harmonization (ICH) on Technical Requirements for Registration of Pharmaceuticals for Human Use. The first one defines and lists validation characteristics (6; Table 1); the second gives more detailed guidance on the protocol and how to perform the validation experiments (7).

These guidelines have also been adopted by the U.S. Food and Drug Administration (FDA), and have been incorporated in the U.S. Pharmacopoeia (USP), replacing the previous general chapter "Validation of Compendial Methods" (8).

Numerous papers have been published, addressing analytical validation and especially validation of chromatographic procedures. Only a limited number, however, deal with the validation of planar chromatographic procedures (9–15).

This paper provides a general survey covering validation of all the wide-ranging applications of planar chromatography in pharmaceutical analysis, the only technique which is used routinely for all types of tests mentioned in Table 1. In addition, acceptance criteria are proposed for the individual validation parameters. These reflect the performance to be expected for the analysis of defined chemical entities and resulting finished products. In the case of more complex analytes—as natural compounds, oligomers, etc.—individual (wider) limits may be acceptable. Definitions follow the terms of the ICH guidelines (6, 7).

Prevalidation Considerations

The process of validating a procedure cannot be separated from its development, as the analyst will not know whether the procedure and its performance parameters are acceptable until validation has been performed. Validation and development therefore may be considered as an iterative process.

Table 1. Types of analytical procedures and required validation characteristics taken from reference 6[a]

Type of analytical procedure Characteristics	Identification	Testing for impurities		Assay Dissolution (measurement only) Content/potency
		Quantitat.	Limit	
Accuracy	–	+	–	+
Precision				
Repeatability	–	+	–	+
Intermediate precision	–	+[b]	–	+[b]
Specificity[c]	+	+	+	+
Detection limit	–	–[d]	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

[a]   – = This characteristic is not normally evaluated; + = this characteristic is normally evaluated.

[b]   In cases where reproducibility has been performed, intermediate precision is not needed.

[c]   Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).

[d]   May be needed in some cases.

However, actual best practice requires a very formalized protocol to be followed. Thus, before outlining this validation protocol and the experimental design involved, it is necessary to make some basic assumptions (16, 17): (*1*) All instrumentation and equipment has been properly qualified. (*2*) All solvents, reagents, plates, and reference standard material have been defined, specified, and tested. (*3*) The analytical procedure has been developed, optimized, and documented. This generally requires prevalidation experiments and robustness checks. (*4*) A validation protocol including acceptance criteria and laying down the statistical approaches necessary to evaluate the data has been agreed upon and signed.

A logical, stepwise approach to analytical validation may then be proposed, as some of the parameters listed in Table 1 can be measured in combined experiments (16, 18).

One of the most vital steps in these prevalidation experiments—or the first step in validation—is the examination of the stability of the target analyte during chromatographic investigation.

In classical TLC/HPTLC, degradation may occur when using sensitive substances during application or development, especially on the highly active polar surface of normal-phase silica gel and by contact with the atmospheric oxygen of the vapor phase.

In OPLC, light and the vapor phase of the eluent are eliminated during the development step (19); however, off-line sample application may degrade oxygen- or light-sensitive substances. Therefore, it is essential for all planar chromatographic techniques to confirm the stability of the target analyte at the end of the chromatographic step.

This can be demonstrated by: (*1*) Two-dimensional separation using the system eluent in both directions. (*2*) Chromatography of sample solutions that have been standing for different periods of time (24, 4, 2, 1, 0.5, 0.1 h) before application. (*3*) Applying sample solutions to the plate at different times

(4, 2, 1, 0.5, <0.1 h) before development. (*4*) Performing critical sample preparation steps using different time periods and evaluating the impurity profiles subsequent to chromatography. This is especially vital for finished pharmaceutical products. (*5*) Evaluating the chromatogram immediately, 0.5, 2, 4, or more hours after derivatization or final drying.

The term "active pharmaceutical ingredient" (API) as proposed by the ICH (20) is used in this paper, rather than the synonyms "bulk drug substance" or "active substance" or the term "impurities" as defined by the ICH Impurity Guideline (21, 22) is used as a general term in this paper, independently of the nature or source.

Acceptance criteria.—The analyte should be stable during development in solution for not less than 1 h, on the sorbent surface before development for not less than 30 min, during sample preparation for not less than 30 min, and on a plate after chromatography for not less than 1 h.

If the stability is not satisfactory, degradation may be suppressed by using antioxidants in solutions (e.g., butylated hydroxy-toluene, ascorbyl palmitate). Sample application can also be performed under N_2 atmosphere, eliminating atmospheric oxygen from the chamber by introducing inert gases, using a sorbent layer with a concentrating (pre-adsorbent) zone, working protected from light, or switching to procedures using reversed-phase separation systems.

Validation

The description of the validation steps according to the ICH requirement outlined here follows the type of analytical procedure rather than the validation parameters.

Identification

Planar chromatography is widely used for identification of APIs in finished products or key components in herbal ex-
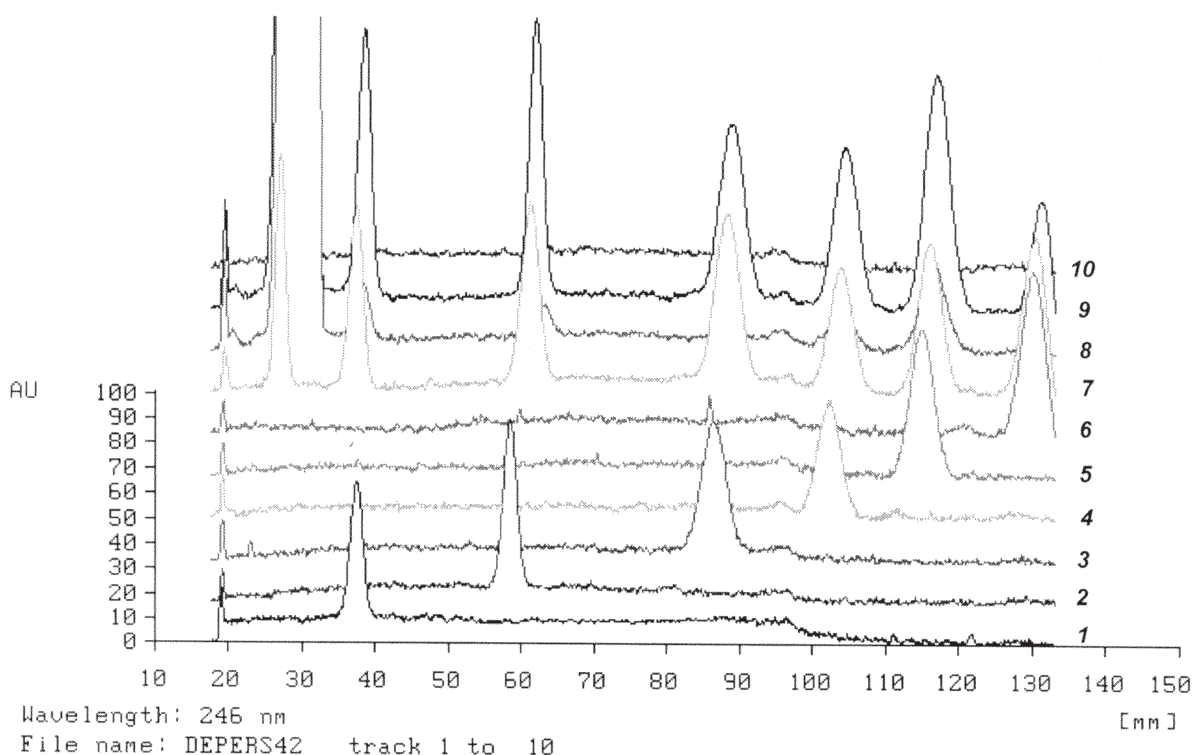
Figure 1.   Specificity of the purity test for mazipredone drug substance: (1)–(6) 0.5–0.5 μg impurities; (7) mixture of 0.5–0.5 μg impurities and mazipredone; (8) 50 μg mazipredone; (9) 50 μg mazipredone spiked with 0.5–0.5 μg impurities; (10) blank.

tracts. Following a suitable sample preparation, if necessary, sample and standard substance(s) are chromatographed simultaneously and derivatized if required. Matrix effects must be eliminated to obtain undisturbed spot-shape and R_f-values.

Specificity.—The validation parameter to be determined is specificity (selectivity). The sample, reference standard substance(s), pure and spiked placebo are chromatographed simultaneously side by side.

Acceptance criterion: adequate separation of the API (or other substances to be identified) from all other components of the sample.

Documentation can be performed using color photographs or video prints. The validation report must contain these documents in original or digitized form. The advantage of the latter approach is the easy and unambiguous compilation of documentation (23).

Testing for Impurities with the Limit Test

In this case, the impurities present are determined mainly by visual comparison of size, color, color after derivatization, and intensity of the spots or bands in the chromatogram of the analyte (API or pharmaceutical product); these are then compared with the spots or bands of a reference standard or of the main component in diluted solutions of the analyte. This simple and fast approach allows the evaluation of 5–10 samples within a few minutes and is widely used as an in-process control and as a pharmacopoeial limit test.

More recent approaches use video densitometry or scanning densitometry for evaluation to increase the reliability of results, especially when levels near to specification limits have to be tested.

Validation parameters to be determined according to the ICH guidelines are specificity and detection limit.

(**a**) *Specificity for known/available impurities*.—In case of an API, the pure substance, its known, available impurities, and the API spiked with the impurities at specification level are chromatographed simultaneously. R_f-values characterizing specificity are determined and documented.

Acceptance criteria: adequate separation of the API from its known impurities and of the impurities from one another, $0.1 \leq R_f \leq 0.9$ (R_f = retardation factor = distance travelled by the center of the spot/peak ÷ distance travelled by the mobile phase front). If video densitometry or scanning densitometry is used for evaluation, R_s (R_s = peak resolution = 1.18 × distance between 2 adjacent spots/peaks ÷ sum of the 2 peaks width at half height) and $A_{0.05}$ [$A_{0.05}$ = asymmetry factor (tailing factor) = the width-ratio of the 2 halves of the peak (front/tail) measured at 5% height] may also be used as acceptance criteria.

In case of a pharmaceutical product, the product (after suitable sample preparation, if necessary), the API(s), the known impurities of the API(s), the product spiked with these impurities, and the pure placebo are chromatographed simultaneously.

Acceptance criteria: adequate separation to allow determination of the known degradation products and/or the known impurities of the API(s), expressed as above (Figure 1).

Impurities originating from synthesis of the API (by-products, precursor molecules, etc.) are controlled by testing the API, as levels can be expected to be unchanged during production of the finished pharmaceutical product. It is therefore not compulsory that the chromatographic system for impurity testing of pharmaceutical products separates all possible impurities of the API (22), but it may be an advantage (24).

(**b**) *Specificity for unknown/unavailable degradation products*.—Especially if the analytical procedure is considered to be stability indicating, validation of specificity (selectivity) based on forced degradation tests must also be performed.

The API or the pharmaceutical product, and in case of a product a placebo too, are subjected to heat stress, humidity, light (UV, Xe) in solid form as well as to acid, base, light (UV, Xe), oxidizing (H_2O_2) or reducing agents (Zn/HCl), and hydrolytic conditions in solution. Stress should lead to 5–10% degradation of the API, if possible. Identification of the stress-degradation products is not compulsory, but knowledge of the main degradation profiles is a main issue of the stability features of the API (Figure 2).

Separation efficiency should also be demonstrated by proving peak purity. Different approaches are possible, but the most simple one is 2-dimensional chromatography using the system eluent in the first direction and an eluent of different selectivity in the second. Other approaches are recording in situ UV, VIS, or IR spectra.

Acceptance criteria: suitable separation and detection of spots of stress degradation products, expressed as above, no new impurity spots detectable in the second dimension of the 2-dimensional separation, or peak purity proven by in situ spectroscopy.

(**c**) *Detection limit (DL)*.—To determine the detection limit, the API (and its impurities, if available) are applied and developed in decreasing quantities at least in duplicate. The

smallest visible quantity determined by at least 3 different analysts is regarded as the DL.

In the case of finished pharmaceutical products, the API and/or its impurities are added to the placebo in decreasing quantities. After sample cleanup and preparation, aliquots of the spiked placebo solutions are chromatographed. Determination and documentation of DL are the same as in case of APIs.

Acceptance criterion: the ICH guidelines on impurities (21, 22) require identification and/or qualification of impurities exceeding an (apparent) level of 0.1%. The validated quantitation limit (QL) is required to be less or equal to 0.05% (50% of the specification limit). For semiquantitative procedures, no acceptance criteria for the QL have been defined; however, taking into consideration the general requirement mentioned above, the DL should be considerably lower than 0.05% (or 50% of the specification limit). A DL of 10–20% of the claimed specification limit is therefore regarded as being sufficient.

An insufficient DL may be improved either by increasing the applied quantity of sample (if no interference or plate overloading phenomena occur) or by using the selective and sensitive pre- or postchromatographic derivatization procedures available in planar chromatography (25).

Testing for Impurities by Using Quantitative Procedures

A quantitative planar chromatographic purity test with quantitative (either by video scan or scanning densitometry) evaluation requires the most comprehensive validation study, covering accuracy, precision, specificity, DL, QL, and linearity and range. Determination of these parameters in the case of an API has been described in detail (10, 14). Validation characteristics can be calculated and characterized using standard statistics and commercially available software.

(**a**) *Specificity for known/available impurities*.—The procedure is the same as described in *Testing for Impurities with the Limit Test* (*Specificity for Known/Available Impurities*).
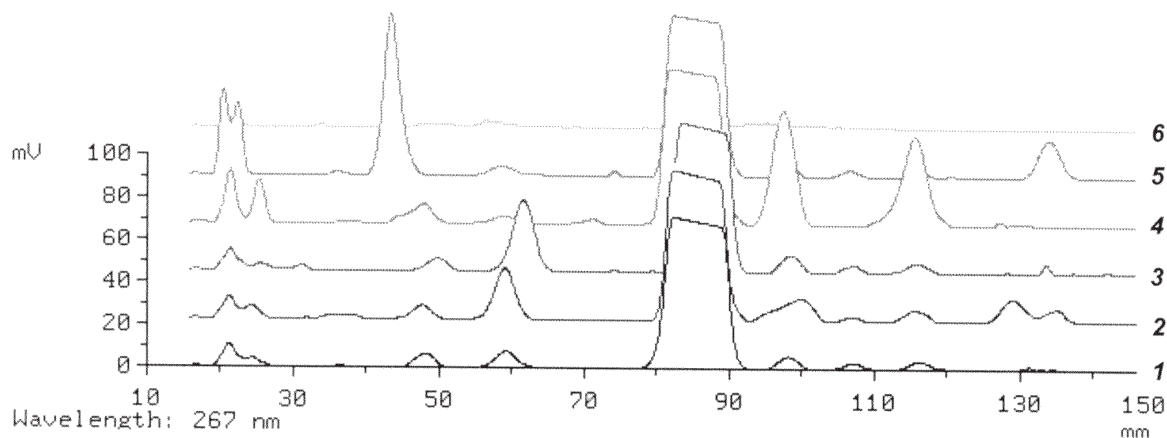

Figure 2. Specificity for unknown degradation products of famotidine drug substance (Stress test): (*1*) untreated famotidine; (*2*) famotidine treated by UV light in solution; (*3*) famotidine treated by oxygen in solution; (*4*) famotidine treated by base in solution; (*5*) famotidine treated by acid in solution; (*6*) blank.
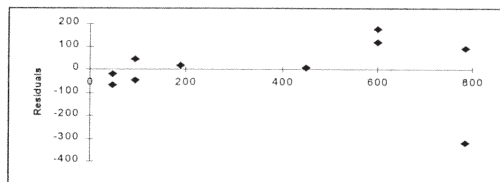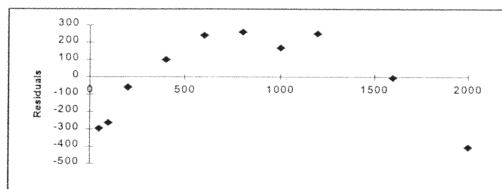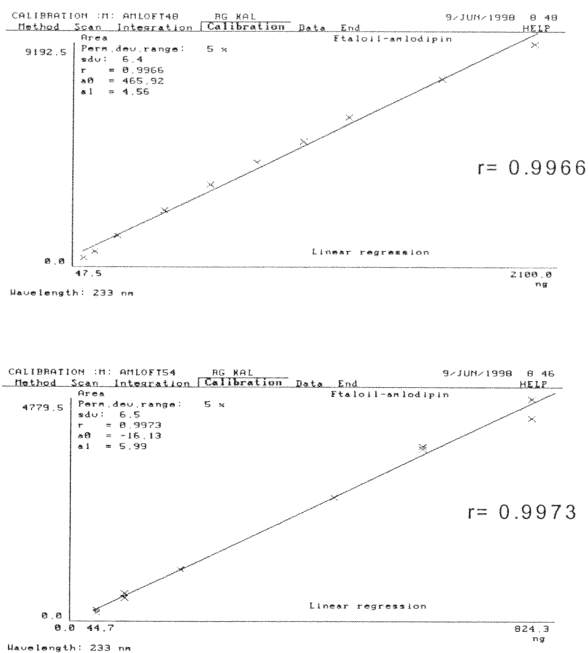
Figure 3. Testing linearity of calibration curves by residuals. (A) Nonlinear calibration in wide range; (B) linear working range. This example shows that the correlation coefficient alone is not suitable to prove linearity.

For characterizing the specificity of the procedure, R_f, R_s, and $A_{0.05}$ ($R_s \geq 1$; $0.8 \leq A_{0.05} \leq 1.2$) values are calculated and determined. Figure 1 shows the densitograms of a specificity test.

(**b**) *Specificity for unknown/unavailable degradation products*.—The procedure is performed as described under *Testing for Impurities with the Limit Test* (*Specificity for Unknown/Unavailable Degradation Products*). Comparison and evaluation of the densitograms of unstressed and degraded samples proves the stability-indicating feature of the analytical procedure. Figure 2 gives an example of a forced degradation experiment.

(**c**) *Linearity and range*.—Unfortunately the term linearity is used in ICH guidelines (6, 7) as well as in the USP (8), although there are numerous analytical techniques or steps in analytical procedures that show or lead to a nonlinear relationship between concentration of analytes in samples and the corresponding measurement signal. In addition, a measurement directly proportional to the analyte quantity should not automatically allow a one-point calibration in routine measurement.

The validation parameters to be determined therefore are not only the mathematical correlation between signal and amount in the selected working range, but also verification of the proposed calibration model in routine analysis.

In planar chromatography, especially in the case of evaluation by scanning in the UV or VIS reflection mode, most calibration functions are nonlinear. The well defined concentration ranges and specification limits in pharmaceutical analyses often make it possible to find and use a quasi-linear calibration function where there is no significantly better fitting of the calibration data with a nonlinear (e.g., polynomial) regression. These quasi-linear regression equations often show an intercept significantly different from zero. Therefore, for routine analytical procedures, a 3-point calibration model must be used, covering the defined working range as described in the draft monograph of the European Pharmacopoeia (5).

If no linear or quasi-linear correlation is obtainable, the calibration should be based on a nonlinear (polynomial, Michaelis-Menten, etc.) regression rather than on a "linear" calibration derived by means of a mathematical transformation (26). Modern software for calculating quantitative results from planar chromatographic data captured either by scanning or by video densitometry offers the possibility of using nonlinear alternatives.

However, for procedures based on nonlinear (e.g., polynomial) regression, we recommend a minimum of 4 independent calibration points (double spotting) in the routine use.

Table 2. Acceptance criteria for linearity in impurity testing

Parameter	Acceptance criteria
Residual plot	No trend
Correlation coefficient	$r \geq 0.99$
y-Axis intercept	$\leq 25\%^a$
RSD of residuals	
Impurity level $\leq 0.5\%$	$\leq 10\%$
Impurity level $\geq 0.5\%$	$\leq 5\%$

[a] When referred to the y value of the regression line (calculated response) for the concentration corresponding to the specification limits of the impurity.

Table 3. Acceptance criteria for precision of impurity testing

$n = 6$	API	Finished product
Repeatability		
Level 0.1–0.2%	RSD ≤20%	RSD ≤20%[a]
Level 0.2–0.5%	RSD ≤10%	RSD ≤10%
Level >0.5%	RSD ≤5%	RSD ≤5%
Intermediate precision	1.5 × RSD of repeatability	1.5 × RSD of repeatability

[a] Higher RSDs may be acceptable.

For proving linearity or basic calibration in the proposed working range, a 10-point calibration graph is constructed from not less than 8 independent and equidistant concentrations, statistically distributed. The highest and the lowest concentration are spotted twice.

Other approaches use 9 independent concentrations, with double spotting (data-pair technique) for each concentration. This basic calibration should be performed for each individual impurity if the procedure is based on reference standards of these impurities or simply for the main component (API) if the quantitation of impurities is based on the comparison with peak area or peak height of the main component.

Residuals of the regression line are plotted against the applied quantities. The calibration function can be regarded as being linear if residuals are distributed around the 0-line at random, without any trend. Figure 3 shows a linear and a nonlinear calibration graph and their residuals calculated by linear regression. This method is simple and convincing, but its disadvantage is the lack of a numerical limit. The residual test can also be useful in testing the adequacy of nonlinear fitting.

The check for linearity can be done using various statistical approaches (e.g., Mandel's test [18] and Fowlis-Scott [14, 27]).

We do emphasize, however, that the linearity of a basic calibration cannot be proven simply by a correlation coefficient close to 1 (28). This is demonstrated in Figure 3. Acceptance criteria are given in Table 2.

Acceptance criterion for linear range: from QL to 120% of the specified limit of the impurity.

If the linearity in the working range is not adequate, a polynomial or other nonlinear regression mode has to be applied. If influences by matrix components (API, formulation components, etc.) have become apparent during development of the procedure, these components must be added to the calibration solutions (*see Prevalidation Considerations*).

(**d**) *Precision.*—This test provides information about the random error of the analytical procedures including sampling and sample work-up. Precision is expressed as relative standard deviation (RSD) of multiple determinations of a homogeneous sample.

In the ICH guidelines describing pharmaceutical analytical validation, precision is defined at 3 levels: repeatability,

intermediate precision, and reproducibility. In the case of repeatability, the same real sample is determined on the same plate by applying at least 6 analytical solutions from individual weighings. This test provides information about the variation caused by sample preparation, sample application, and evaluation within one analytical run and within a short period of time.

Intermediate precision is determined by analyzing the same real sample on different days, by different analysts, if possible using a different quality of chemicals and different equipment in the same laboratory. This parameter describes the effect of different experimental and environmental conditions on the variability of the result. Table 3 shows acceptance criteria.

The ICH guidelines give no guidance on how to determine intermediate precision. Two different approaches are most common: (*1*) Repeatability test runs are performed on different days with a different combination of analyst, equipment, and in the same laboratory. Mean and RSD values of both sets of data are then compared. (*2*) At least 6 replicate determinations are performed on different days with a different combination of analyst, equipment, and in the same laboratory. RSD value for this set of data is then calculated. Table 3 shows acceptance criteria.

Reproducibility describes the analytical variability between different laboratories at different sites. It is determined by interlaboratory tests (round robin tests) and is not a compulsory part of the analytical validation in pharmaceutical analysis.

(**e**) *Accuracy.*—Accuracy (IUPAC: trueness) expresses the closeness of agreement between the value which is accepted as a conventional true value or an accepted reference value and the result found. This test allows the determination of possible bias.
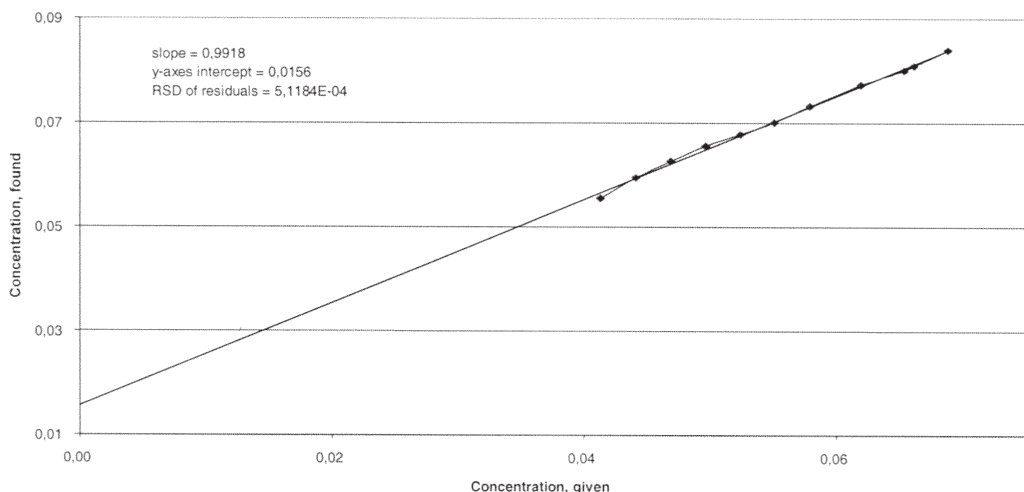
The accuracy of the determination of impurities in APIs is tested by triplicate analysis of solutions of the analyte spiked with 3 different concentrations of the available impurities. The recovery is calculated as a percentage value of the added amount (Table 4).

In the case of finished product testing, determination of the accuracy is even more important as it verifies the quality of the delicate sample preparation step.
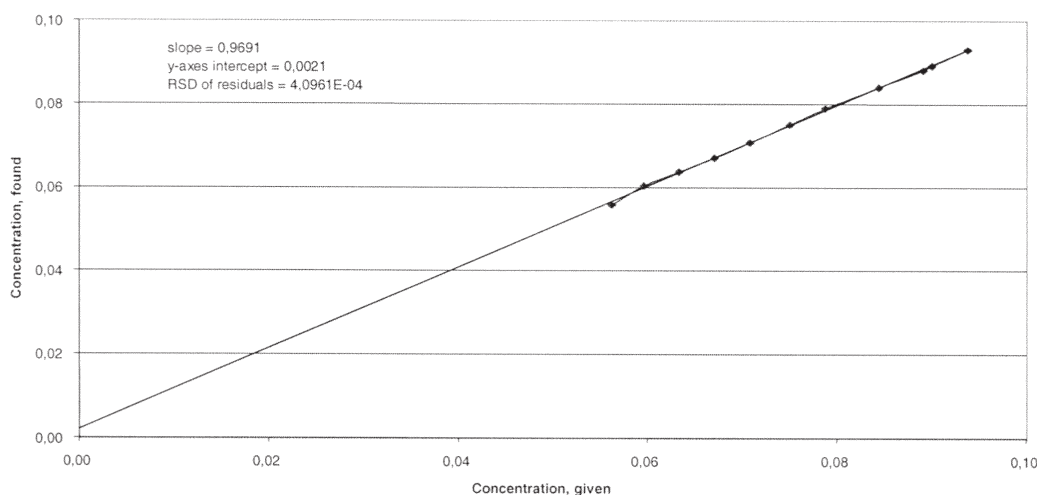
Recovery experiments are difficult to perform as expected degradation products, due to their low concentration, cannot be added to the product or the pure placebo in solid form, but only as aliquot solutions. These aliquots may be dried to the solid surface or mixed into the nonsolid form (cream, injection, etc.) of the finished products. The recovered quantities of the added

Table 4. Acceptance criteria for accuracy of impurity testing of APIs

$n = 3$, 3 Concentrations	Recovery, %	RSD of recoveries, %
Impurity level ≤0.5%	80–120	≤10
Impurity level ≥0.5%	90–110	≤5

slope = 0,9918
y-axes intercept = 0,0156
RSD of residuals = 5,1184E-04

A

slope = 0,9691
y-axes intercept = 0,0021
RSD of residuals = 4,0961E-04

B

Figure 4. Recovery function showing (A) systematic (matrix) effect and (B) no systematic (matrix) effect.

impurities are determined as described above by performing the whole procedure, including sample preparation.

Acceptance criteria for impurity determinations in finished products are close to those given in Table 3 for APIs. Wider ranges may be acceptable in certain cases.

Accuracy may be alternatively proven by construction of a recovery function consisting of at least 6 concentrations covering the working range (for impurities, from QL to 120% of specified level).

If the "found concentrations" are plotted on the ordinate versus the original calibration concentrations on the abscissa,

a recovery curve is obtained. In the ideal case, this recovery function is a line with intercept = 0 and the slope = 1. The recovery function can be accepted if the given confidence interval of the intercept includes the origin and that of the slope includes 1 (generally, $x = 0.05$, $p = 95\%$). For further evaluation, *see* ref. 18. Figure 4 shows recovery functions with and without matrix effects.

(**f**) *Detection limit and quantitation limit*.—Although the determination of DL is not compulsory in the validation of a quantitative impurity test, knowing this data can be useful in establishing the impurity profiles of substances.

Table 5. DL and QL for impurity testing (APIs and finished products)

Parameter	Definition
DL	Signal-to-noise ratio = 3:1
QL	Signal-to-noise ratio = 10:1
	RSD $\leq$10–20%
	$n \geq 5$

For determination of DL and QL, solutions of the expected impurities and the main component (for APIs) or the matrix including API (for finished products; $n \geq 3$) are applied in decreasing quantities, in triplicate. The same volume of the pure solvent as a blank is also applied. After development, a calibration graph is constructed plotting the peak heights or areas against the applied quantities of the substances investigated. DL and QL are calculated based on the signal-to-noise ratio (*see* Table 5; 6, 7).

An alternative procedure for the calculation of QL is the Eurachem approach. In this case, solutions of decreasing concentrations are applied at least 5 times each and the RSD value of the resulting peak areas or peak heights are plotted against the applied quantities (Figure 5). The amount of the QL can then be determined from the resulting graph, based on predetermined RSDs. Generally, the quantity defined by an RSD value of 10% is considered the QL for impurities in APIs and 15% in finished products.
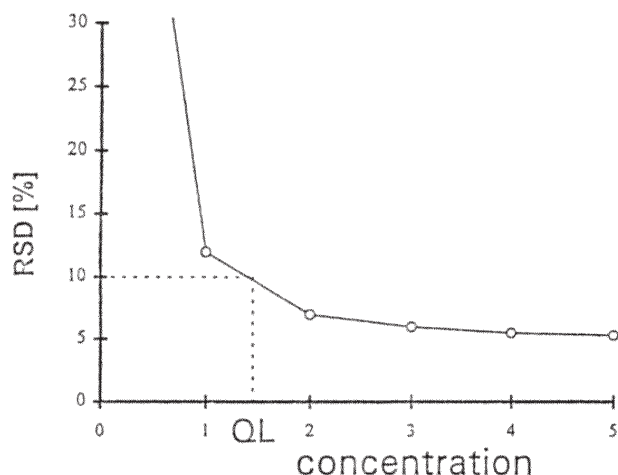

Figure 5. Eurachem approach for determination of QL. Apply analyte (with matrix) in decreasing quantities ($n \geq 5$, random spotting). Plot standard deviation of peak areas or heights against concentration. Determine lowest concentration for which RSD is acceptable (e.g., $\pm$10%).

Further approaches based on statistical models using the calibration function may be used for calculation of DL and QL (*see* Table 6; 6, 7, 18, 29, 30).

Assay

(**a**) *Specificity.*—The procedure is the same as described in *Testing for Impurities with the Limit Test* (*Specificity for Known/Available Impurities*) and *Testing for Impurities by Using Quantitative Procedures* (*Specificity for Known/Available Impurities*), except that the focus is on the API and not the impurities.

Verifying peak purity (either by in situ spectroscopy or subsequent to derivatization) is an additional proof of specificity.

(**b**) *Linearity and range.*—The procedure is the same as outlined in *Testing for Impurities Using Quantitative Procedures* (*Linearity and Range*; *see* also Table 7).

If the linearity in the working range (*see* Table 8) is not adequate, a polynomial or other nonlinear regression mode must be applied (31).

(**c**) *Precision.*—The procedure is the same as described in *Testing for Impurities by Using Quantitative Procedures* (*Precision*; *see* also Table 9).

(**d**) *Accuracy.*—For the assay of an API, accuracy measurements are performed by comparison of the results with the analysis of a standard reference material or by comparison with a second, well-characterized method.

For the assay of a drug product, accuracy is evaluated by analyzing synthetic mixtures spiked with known quantities of components to be determined [as described in *Testing for Impurities by Using Quantitative Procedures* (*Accuracy*)].

If a guaranteed analyte-free matrix is not available, standard addition experiments must be used. Because new unknowns and inexactitudes are introduced during the preparation of tablets, the validation of analytical procedures for tablets is based on recovery experiments with mixed aliquots of analyte and matrix components.

To document accuracy, the ICH guideline on methodology (7) recommends collecting data from a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range [for example, 3 concentrations, 3 replicates each; *see Testing for Impurities by Using Quantitative Procedures* (*Accuracy*)].

The data should be reported as the percentage recovery of the known, added amount, or as the difference between the mean and true value with confidence intervals (Table 10).

For evaluation of recovery functions, *see Testing for Impurities by Using Quantitative Procedures* (*Accuracy*) and Figure 4.

Table 6. Acceptance criteria for DL and QL in impurity testing (100% = specified limit for impurity)

Substance	DL, %	QL, %
API	$\leq$10	$\leq$20
Finished product	$\leq$20	$\leq$50

Table 7.   Acceptance criteria for linearity for assays/content uniformity measurements

Parameter	Acceptance criteria
Residual plot	No trend
Correlation coefficient	r $\geq$ 0.998
y-Axis intercept	$\leq$ 2%[a]
RSD of residuals	$\leq$ 1.5%

[a] When referred to the y value of the regression line (calculated response) for the concentration corresponding to the specification limits of the assay.

Robustness

It may be useful to explain again the difference between ruggedness and robustness. Ruggedness according to USP 23 (8) is defined as the degree of scatter of test results obtained by analysis of the same sample under a variety of conditions in different laboratories. This variability is termed reproducibility in the ICH guidelines (6, 7).

Robustness however, is defined as the capacity of a method (better: procedure) to remain unaffected by small, deliberate variations in method parameters (6, 7), as they may occur in one laboratory during long-term routine use.

As stated in the ICH guidelines, robustness is not a part of the formal validation process but should be considered early during method/procedure development.

A robustness test should not be confused with optimization of the procedure. Optimization is a part of the development of the procedure, whereas, robustness testing challenges the "stability" of the established analytical procedure.

There are many chromatographic and environmental parameters that might have an effect on planar chromatographic performance (32). Some selected parameters for robustness testing are as follows: temperature, relative humidity, sorbent type/supplier, mobile phase composition, geometry of chamber, chamber saturation, and measuring wavelength.

Experimental design may be helpful in performing robustness tests with only a reasonable number of experiments, as described above for a quantitative TLC and OPLC test in detail (14, 15).

Table 8.   Acceptance criteria for linear range

Parameter	Acceptance criteria
Assay	80–120% Label claim
Content uniformity	70–130% Label claim
Dissolution testing	± 20% of Upper/lower limit

Proof of the stability of the analyte under chromatographic or sample preparation conditions has been described above (*see Prevalidation Considerations*).

Quality Assurance in Routine Work

In planar chromatography, a new, original sorbent layer is used for every analysis in the separation process. Therefore, system suitability tests have less importance than in HPLC, where the separation is heavily influenced by the actual condition of the sorbent.

Nevertheless, some system suitability tests must also be performed in planar chromatography to control the effect of any unexpected change in experimental or environmental conditions and to ensure the quality of the plate or batch of plates used.

HPLC and gas chromatographic (GC) procedures require pre-analytical system suitability testing runs to demonstrate the actual performance of the system. In planar chromatography, samples and reference standards are analyzed on the same plate simultaneously, side by side ("in-system control"). The system suitability testing, therefore, is done by simply spotting a control sample or reference substance onto the plate.

The resolution can be controlled by a sample pair having close R_f values: the resolution of the chromatogram can be accepted if this critical sample pair is well separated.

For testing the sensitivity of the procedure in impurity testing, an amount of the analyte representing a quantity near the DL is spotted onto the plate in each purity test. If this quantity can be evaluated in the developed chromatogram, the sensitivity of the actual purity test is considered as adequate.

In addition, the parallel testing of control samples does not only allow the performance of the chromatographic step to be evaluated, but also a system of control charts to be run (14, 18, 33). This enables the analyst to control the overall analytical performance and evaluate potential out-of-specification quantitative results.

Table 9.   Acceptance criteria for precision of assay, content uniformity, and dissolution testing (measurement only)

Type of determination	Precision level	Acceptance criteria
Assay (finished product)	Repeatability	RSD $\leq$ 2% ($n \geq 6$[a])
	Intermediate precision	RSD $\leq$ 3% ($n \geq 6$)
Content uniformity (finished product)	Repeatability	RSD $\leq$ 2% ($n \geq 6$)
	Intermediate precision	RSD $\leq$ 3% ($n \geq 6$)
Dissolution rate (measurement only)	Repeatability	RSD $\leq$ 3% ($n \geq 6$)
	Intermediate precision	Depends on project

[a] $n \geq 6$ Replicate individual analysis of the same homogeneous sample including 6 weighings and sample preparations, each with double spotting.

Table 10. Acceptance criteria for accuracy of assay, content uniformity, and dissolution testing (measurement only)

Type of determination	Acceptance criteria
Assay API	Mean of 6 determinations = ± 2% of nominal value[a]
Assay API in finished product	Recovery 95–105%
	RSD of recovery ≤ 3%

[a] 95% Confidence level.

On each plate, besides the samples to be determined, a control sample is determined simultaneously in duplicate. Control charts can then be compiled after collecting not less than 20 duplicates. The most common charts are the average chart and the range chart (Shewhart charts).

On the average chart, the results measured for the control sample are plotted against the serial number of measurements. If these data are normally distributed, 99.7% of these results should be within the range of $\bar{x} \pm 3s$, where $\bar{x}$ is the mean and s is the standard deviation of the mean of all results determined for the control samples. Therefore, $\bar{x} \pm 3s$ are the action limits of the mean chart. If the actual result of the control sample is outside of these limits, the analytical process must be considered out of control and the analytical result of the real sample cannot be accepted. Tighter warning limits ($\bar{x} \pm 2s$) help to monitor the analytical work. Average charts thus provide information on the accuracy of the actual measurement.

On the range chart, the difference of the 2 results of the duplicate measurement of the control sample is plotted against the serial number of measurement. The limit of this chart is calculated by multiplying the mean range with a tabulated value. If the difference actually determined for the control sample is outside of this limit, the analysis must be repeated. The range chart provides information on the precision of the analytical process.

Further charts can be compiled by plotting values derived from calculations from the data of the control sample. The points of the cusum (cumulative sum) chart correspond to the cumulative sum of the difference between the actual values and the mean value.

In the moving average chart, the plotted average values contain the actual value and also the previous ones (3 or 5); these, however, are weighted differently.

Because the cusum and the moving average charts smooth the raw, measured data, they are excellent tools for discovering potential trends over a period of time. They can be caused for example by the slow degradation of the control sample. The quality of a control chart heavily depends on the quality of the reference sample.

Table 11 lists some of the most common types of control charts.

Figure 6 shows control charts for data derived by analyzing a famotidine control sample with the actual test runs included.

Conclusions

Contemporary planar chromatography nowadays is a chromatographic technique that fully complies with Good Manufacturing Practices (GMPs). Results can be objectively documented both in computerized form and on hard copies—qualitative and semiquantitative testing as a video picture or video densitogram and quantitative assays or tests as a densitogram.

Table 11. Examples of some commonly used control charts

| Type of control chart	Data entry	Suitable for detecting	Required analyses
Average chart	Mean value (and standard deviation), control and warning limits calculated/defined from previous period	Gross errors (deviation from the mean); systematic errors (trends, changes in the mean); random errors (scatter of individual values), generally for accuracy control	Control sample analyses in duplicate
Range chart	Range from the control analyses mean range, control limits calculated/defined from the previous period	Precision control	At least 2 determinations for control samples
Cusum chart	Calculated sum of the deviations from one target value, e.g., mean of an assay; reference value calculated/defined from the previous period	Inaccuracy; drift in data series; trends over time	Control sample analyses
Moving average chart	Average of a given number of mean values, e.g., $n = 3$ or 5 from the previous period, last entry includes actual value	Drift in data series; prediction of future mean value; trends over time	Control sample analyses
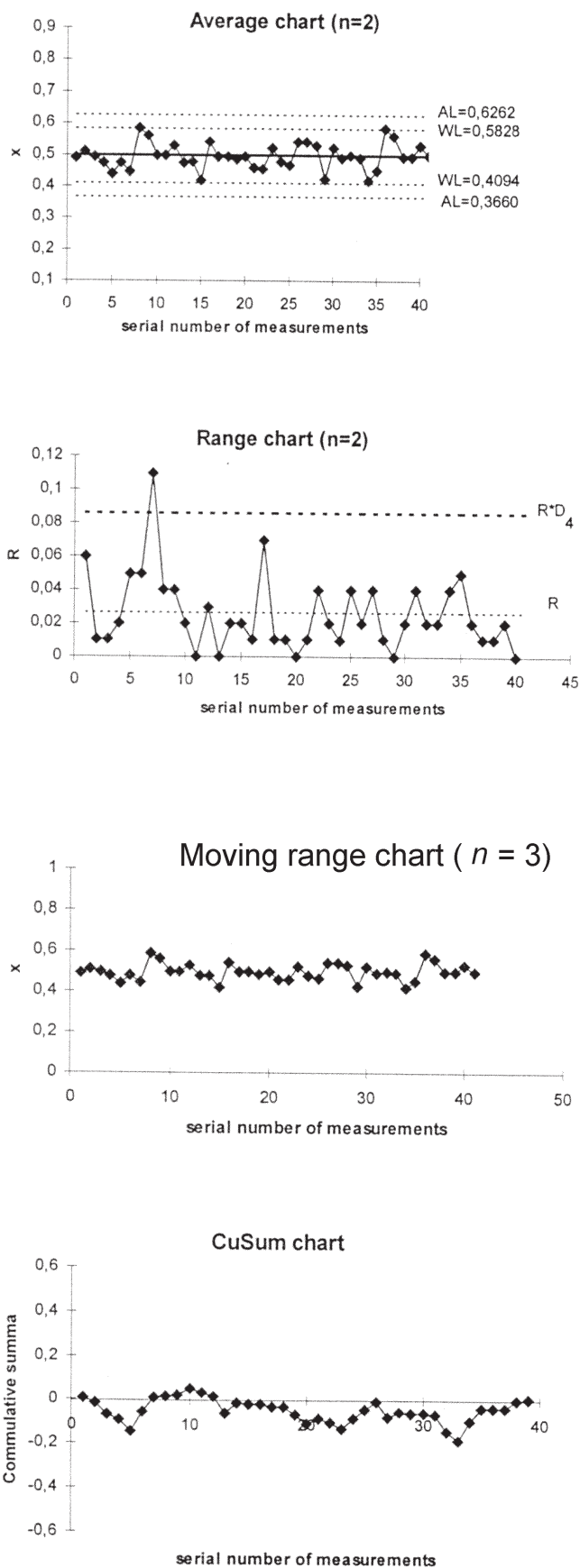
Figure 6.   Control charts for control samples of a TLC purity test (famotidine).

Validation requirements for planar chromatographic analytical procedures are highly diversified, depending on the actual type of test, as planar chromatography can be used in a wide range of applications from a simple qualitative identification to a quantitative assay or purity test.

When choosing the validation characteristics to be determined, instructions provided in the corresponding ICH guidelines were taken into consideration. The acceptance criteria for every characteristic proposed and presented in this work are based on many years of experience on the part of the authors using planar chromatography in the field of pharmaceutical analysis. As can be seen from the acceptance criteria, quantitative planar chromatography performed with suitable care and expertise can provide results that can compete with those derived from HPLC in respect to specificity, accuracy, and precision.

Because sensitive substances may easily degrade on the highly active polar surface of the sorbent layer, the control of the stability of the analyte during sample preparation and the chromatographic process itself is strongly recommended when performing planar chromatography.

Although performing robustness tests is not compulsory, they provide useful information concerning the transferability and long-term stability of procedures. It is worthwhile performing, particularly in cases when procedures are intended to be used in different laboratories for a long time, e.g., in release or stability testing.

In routine practice, it is strongly recommended that a validated procedure be monitored by testing control samples with each test run. The suitable selectivity of separation, the sensitivity of evaluation, and the accuracy and precision of the quantitative determination can then be checked by evaluating the results of the control samples.

We hope that this work, originating from an international effort, may positively stimulate the standardization of planar chromatography in pharmaceutical quality control to the advantage of economical analysis.

References

(1) Volford, O., Vámos, J., & Takács, M. (1996) *Int. J. Pharm.* **137**, 111–115

(2) Ferenczi-Fodor, K., Mahó, S., Pap-Sziklay, Z., Török, I., & Borka, L. (1997) *Pharmeuropa* **9**, 736–742

(3) Altorfer, H.R., Christiansen, C.P., Dammertz, W., Lang, J., & Renger, B. (1996) *Pharmeuropa* **8**, 172–174

(4) Renger, B. (1998) *J. AOAC Int.* **81**, 333–339

(5) Monographie 2.2.7: Thin-Layer Chromatography (1998) European Pharmacopoeia Supplement, 3rd Ed., 1999 European Pharmacopoeia Commission, Strasbourg, France

(6) ICH Guideline Q2A, Validation of Analytical Procedures: Definition and Terminology (CPMP III/5626/94) (March 1995) Geneva, Switzerland

(7) ICH Guideline Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH/281/95) (November 1996) Geneva, Switzerland

(8) U.S. Pharmacopoeia 23, Supplement 4 (1996) U.S. Pharmacopoeial Convention, Inc., Rockville, MD, pp 3261–3262

(9) Szepesi, G. (1993) *J. Planar Chromatogr.* **6**, 187–197

(10) Ferenczi-Fodor, K., Végh, Z., & Pap-Sziklay, Z. (1993) *J. Planar Chromatogr.* **6**, 198–203

(11) Sun, S.W., & Fabre, H. (1994) *J. Liq. Chromatogr.* **17**, 433–445

(12) Sun, S.W., Fabre, H., & Maillols, H. (1994) *J. Liq. Chromatogr.* **17**, 2495–2509

(13) Renger, B., Jehle, H., Fischer, M., & Funk, W. (1995) *J. Planar Chromatogr.* **8**, 269–278

(14) Ferenczi-Fodor, K., Nagy-Turák, A., & Végh, Z. (1995) *J. Planar Chromatogr.* **8**, 349–356

(15) Nagy-Turák, A., Végh, Z., & Ferenczi-Fodor, K. (1995) *J. Planar Chromatogr.* **8**, 188–193

(16) Huber, L. (1998) *LC:GC Int.* **11**, 96–105

(17) Swartz, M.E., & Krull March, I.S. (1998) *Pharm. Techn.* 104–119

(18) Funk, W., Dammann, V., & Donnevert, G. (1995) *Quality Assurance in Analytical Chemistry,* VCH, Weinheim, Germany

(19) Mincsovics, E., Ferenczi-Fodor, K., & Tyihák, E. (1996) in *Handbook of Thin-Layer Chromatography,* 2nd Ed., J. Sherma & B. Fried (Eds), Marcel Dekker, New York, NY, pp 171–203

(20) ICH Guideline Q7A (1999) "Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients," Draft 4, Bruxelles, Belgium

(21) ICH Guideline Q3A (1995) "Impurities in New Drug Substances" (CPMP/ICH/142/95), Geneva, Switzerland

(22) ICH Guideline Q3B (1996) "Impurities in New Medicinal Products" (CPMP/ICH/282/95), Geneva, Switzerland

(23) Végh, Z., Wiszkidenszky, A., Narancsik, A., & Ferenczi-Fodor, K. (1998) in *Proceedings of the 10th Int. Symp. Instrumental Planar Chromatography, Visegrád,* S. Nyiredy, B. Szabady, R.E. Kaiser, & A. Studer (Eds), Research Institute for Medicinal Plants, Budakalász, Hungary

(24) Rabonhons, M. (1997) *Conference documentation,* 6th Int. Conf. Controlling Impurities in Bulk Drug Substances and Products, London, UK

(25) Jork, H., Funk, W., Fischer, W., & Wimmer, H. (1990 and 1994) in *Thin-Layer Chromatography: Reagents and Detection Methodes, Vols. 1a & 1b*, VCH, Weinheim, Germany

(26) Ebel, S., & Rost, D. (1980) *Arch. Pharm. Weinheim* **313**, 337–343

(27) Fowlis L.A., & Scott, R.P. (1963) *J. Chromatogr.* **11**, 1–10

(28) Analytical Methods Committee, London Royal Society of Chemistry (1988) *Analyst* **113**, 1469–1471

(29) Vogelsang, J., & Hadrich, J. (1998) *Accred. Qual. Assur.* **3**, 242–255

(30) Kaus, R. (1998) *Accred. Qual. Assur.* **3**, 150–154

(31) Horwitz, W. (1998) *Inside Lab. Management* **2**, 4–5

(32) Szepesi, G. (1993) *J. Planar Chromatogr.* **6**, 259–268

(33) Massart, D.L., Vandeginste, B.G.M., Deming, S.N., Michotte, Y., & Kaufman, L. (1998) *Chemometrics: A Textbook*, Elsevier, Amsterdam, The Netherlands p. 95