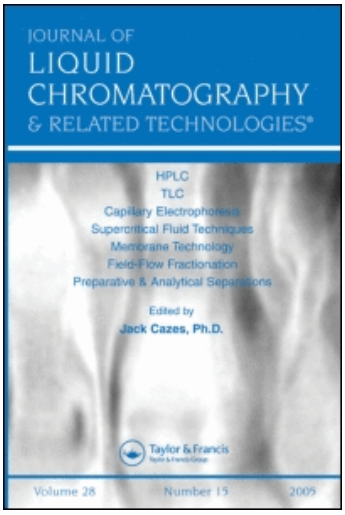

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Liquid Chromatographic Considerations for High Sensitivity Impurity and Stability Testing of Pharmaceuticals

Michael W. Dong[a]; Peter V. Passalacqua[a]; Dilip R. Choudhury[b]
[a] The Perkin-Elmer Corporation, Norwalk, Connecticut [b] Vicks Research Center One Fair Mill Crossing, Shelton, Connecticut

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LIQUID CHROMATOGRAPHIC CONSIDERATIONS FOR HIGH SENSITIVITY IMPURITY AND STABILITY TESTING OF PHARMACEUTICALS

MICHAEL W. DONG[1], PETER V. PASSALACQUA[1], AND DILIP R. CHOUDHURY[2]
[1]*The Perkin-Elmer Corporation*
761 Main Avenue
Norwalk, Connecticut 06859-0250
[2]*Vicks Research Center*
One Fair Mill Crossing
Shelton, Connecticut 06484

ABSTRACT

The use of liquid chromatography for purity and stability testing of bulk and formulated pharmaceuticals is reviewed with particular regard to method development and validation. Recently published USP validation guidelines for both the main drug component and its impurities are also discussed with emphasis on the use of high sensitivity diode array detection and modern chromatographic software. Included in the discussion are instrumental factors and operating conditions for optimum analytical performance.

INTRODUCTION

Liquid Chromatography (LC) is the premier method for impurity assays of bulk drugs and stability indicating assays of pharmaceutical products (1-3). Since drug safety is critically related to drug purity, enormous effort is expended by the pharmaceutical industry to minimize impurities in bulk drugs and to control degradation in the formulated products (4). This

effort begins at the initial stages of pharmaceutical development and continues through the quality control of the final products. Impurity assays by LC are developed and validated to support the manufacturing process, for release assays of each lot of bulk materials, and for stability studies of the formulations (to establish product shelf life). These methods are documented meticulously in "Investigational New Drug (IND)" and "New Drug Applications (NDA)" for subsequent approval by regulatory agencies (5).

This paper reviews the use of LC in stability indicating assays of bulk and formulated pharmaceuticals. Specific requirements for these assays are discussed in light of the new United States Pharmacopeia (USP) guidelines for method validation (6). Since impurity levels are often very low (<0.5%) in most bulk pharmaceuticals, the use of a high sensitivity detector is desirable. In this study, the performance of a high sensitivity LC system is documented to illustrate the use of diode array detection and modern chromatography software for methods development and validation.

EXPERIMENTAL

Equipment

The LC system used in this study consisted of the Model 250 binary LC pump, model 7125 manual injector or an ISS-100 autosampler, the LC-235 diode array detector, the OMEGA-2 Chromatographic Workstation and the GP-100 printer/plotter. An optional software package (OMEGA 235) which allows simultaneous data handling, spectral archiving and manipulation was also used. All LC equipment was from The Perkin-Elmer Corporation (Norwalk, CT). Performance characteristics of each system component have been documented elsewhere (7-9).

Materials and Methods

Chemicals and reagents. Reference standards, chlorthalidone, and its hydrolysis product (4'-chloro-3'-sulfamoyl-2-benzophenone carboxylic acid) were obtained from USP Reference Standard, Inc., Rockville, MD. Chlorthalidone tablets were purchased from a local pharmacy during October of 1989. Other chemicals, organic solvents and reagents were obtained from Aldrich Chemicals (Milwaukee, WI), EM Science (Cherry Hill, NJ) and Fisher Scientific (Fairlawn, NJ). Water was filtered and purified by passage through mixed-bed, ion exchange and activated charcoal cartridges.

Sample preparation and LC analysis procedures. Chlorthalidone tablets were extracted and analyzed according to procedures published in the USP (6) and JAOAC (10). The LC conditions used for the analysis of bulk and chlorthalidone tablets were:

Column:	Pecosphere-C CR C18 (33 x 4.6 mm i.d.)
Mobile Phase:	Methanol-water-acetic acid (35:65:1)
Flow Rate:	2 mL/min
Detection:	UV @ 235 nm

RESULTS AND DISCUSSION

LC methods for stability indicating assays of pharmaceuticals share several common characteristics.

- Analysis is performed mostly with isocratic reversed-phase LC.
- Analytes are generally 1-2 major components and up to several impurities (<0.5%).
- Resolution, accuracy, precision, reproducibility and sensitivity are primary concerns.
- Methods are validated according to regulatory guidelines.

Since most precription drug formulations have a single active ingredient, the LC chromatogram usually consists of one major analyte peak (1,3). For multi-ingredient products or assay

TABLE 1

Analytical Parameters for Method Validation

Analytical Performance Parameter	Assay Cat. I	Assay Cat. II	
		Quant.	Limit
Precision	Yes	Yes	No
Accuracy	Yes	Yes	*
Limit of Detection	Yes	No	Yes
Limit of Quantitation	Yes	Yes	No
Selectivity	Yes	Yes	Yes
Range	Yes	Yes	*
Linearity	Yes	Yes	No
Ruggedness	Yes	Yes	Yes

* Maybe. From USP XXII, Validation of Compendial Methods, p. 1710.

NOTE: Assay Category I is for the main component in the bulk (or active ingredient in the finished products). Assay Category II is for impurities in the bulk drug (or degradation products in the finished dosage forms).

methods using internal standardization, the number of main component peaks might increase to 3 or 4. Excipients may contribute peaks although they are either eliminated in sample preparation (for solid dosage forms) or in many cases eluted with the solvent front. While the analysis of major components is generally straight-forward, the simultaneous assay of impurities places more stringent demands on the skill of the analyst, the efficiency and selectivity of the column and the sensitivity of the detector. In methods development, separation of the parent drug from all potential degradation products, excipients, process precursors and process impurities must be demonstrated to establish a so-called "specific and stability indicating assay".

Isocratic analysis by reversed-phase LC and UV detection constitutes the norm for a stability indicating assay. To verify

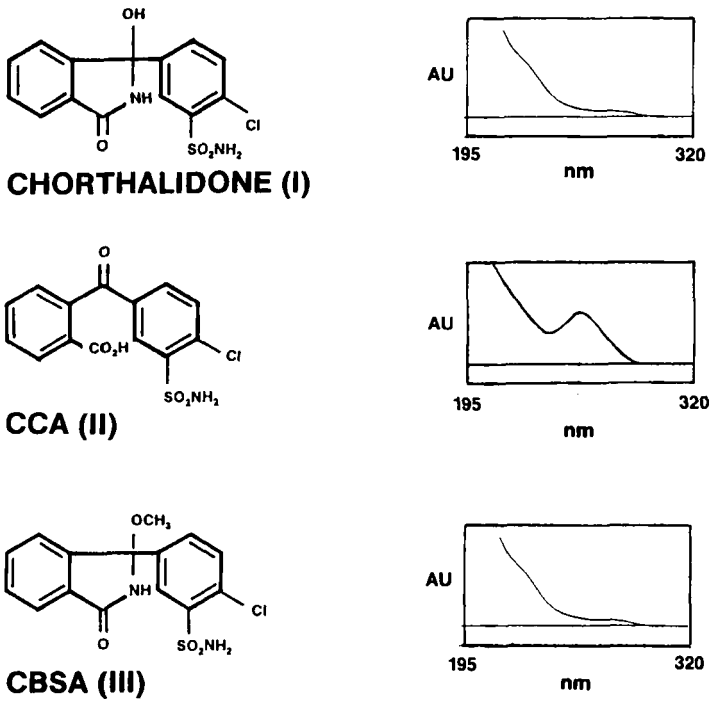

FIGURE 1. Chemical structures of chlorthalidone, CCA (hydrolysis product) and CBSA (degradation product) and their respective UV spectra.

that all accuracy, precision, sensitivity and selectivity requirements are met, the U.S. Pharmacopeia (6) has published specific guidelines for method validation (Table 1). These guidelines clearly differentiate assays of the major drug component (Assay Category I) and impurities (Assay Category II). Implications for each analytical parameter are discussed in detail in the method validation section.

In this study, we used both bulk and formulated chlorthalidone, (a diuretic/antihypertensive agent) to illustrate

the many aspects of LC methods development and validation. Figure 1 shows the chemical structures of chlorthalidone, its hydrolysis product, 4'-chloro-3'-sulfamoyl-2-benzophenone carboxylic acid or (CCA), and a potential impurity, 2-chloro-5-(1-methoxy-3-oxo-1-isoindoliny)benzenesulfonamide or (CBSA), which is formed by the reaction with methanol under acidic conditions (11). The respective UV spectra of the three compounds are included. Note that the UV spectra for chlorthalidone and CBSA are similar while the spectrum of CCA is substantially different because the chromophoric structure is not retained during hydrolysis. Since many drugs have distinctive aromatic structures, UV spectral data can be used effectively for peak identification and purity assessment. These aspects are discussed in the next section.

Diode Array Detection for Methods Development

Diode array detectors (DAD) with their rapid scanning capability can facilitate the development and validation of LC assays (2, 8, 12). Spectral data, conveniently obtained from DADs, effectively supplement the retention time data for peak identification (8). Furthermore, sophisticated spectral manipulation often furnishes useful information on peak purity assessment (13). Table 2 lists several techniques for assessing peak identity and purity in DADs. Their utility in methods development is illustrated in Figures 2, 3 and 6.

Figure 2 (lower trace) shows the chromatogram of pure chlorthalidone at 235 nm. Note that the peak is highly gaussian and no impurity peaks are observable in the chromatogram at 0.5 AUFS. However, a number of questions remain. Is the peak chlorthalidone? Is it pure? Are there any coeluting impurities

TABLE 2

Techniques for Assessing Peak Identity and Purity

Technique		Utility
Spectral Data		
Overlay :	Sample vs standard	Peak purity and identity
	Upslope vs downslope	Peak purity
λ_{max}		Peak identification
Numerical Comparison (Purity Index)		Peak purity
Ratiogram		
Shape		Peak purity
Ratio		Peak identity
Absorbance Profile Map		Selection of detection wavelength Overall sample profile

under the main peak? Figure 2 illustrates how a DAD can provide clues to these questions. First, annotation of a wavelength maximum at 274 nm gives a strong indication of peak identity. Second, annotation of the purity index of 1.0 gives a strong indication of peak purity (13). In addition, since the ratio between two monitoring wavelengths does not change for a pure peak, a flat-topped ratiogram (shown in the upper trace of Figure 2) further confirms peak purity. The ratio value (2.5 for chlorthalidone) can also be used as an identification aid (3).

For impurity assays, the LC chromatogram should be recorded in two ways: first, at low sensitivity (high AUFS) to ensure that the main component does not saturate the absorbance detector (Figure 2, lower trace), and second, at high sensitivity to reveal the presence of low level impurities. Using an analog

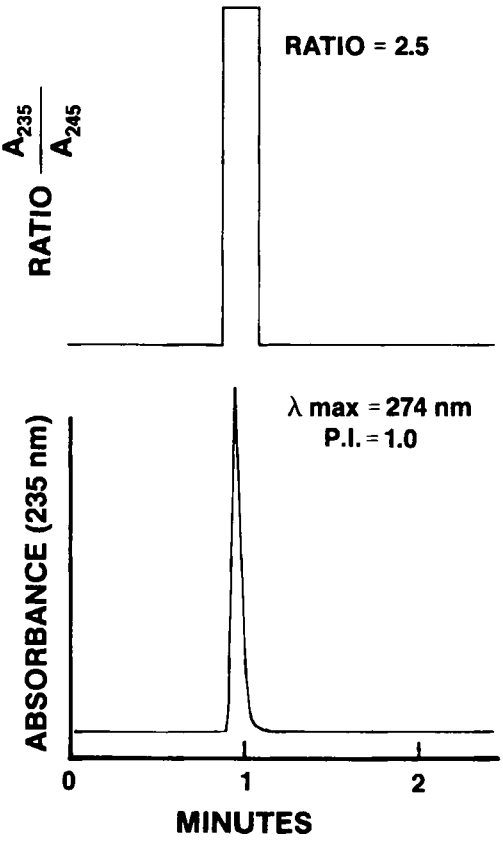

FIGURE 2. Dual channel plot showing a chromatogram of a pure bulk chlorthalidone (0.5 AUFS @ 235 nm) with automated annotation of wavelength maximum (274 nm) and purity index (P.I. = 1.0) . The upper trace shows a ratiogram of 235 nm/245 nm. See the Experimental section in the text for LC conditions.

recorder, this might entail at least two injections into the liquid chromatograph. However, using a data system with raw data storage, a stored chromatogram from a single injection can be displayed or plotted in many ways to visually enhance any region of the chromatogram. Figure 3 shows the screen display of the

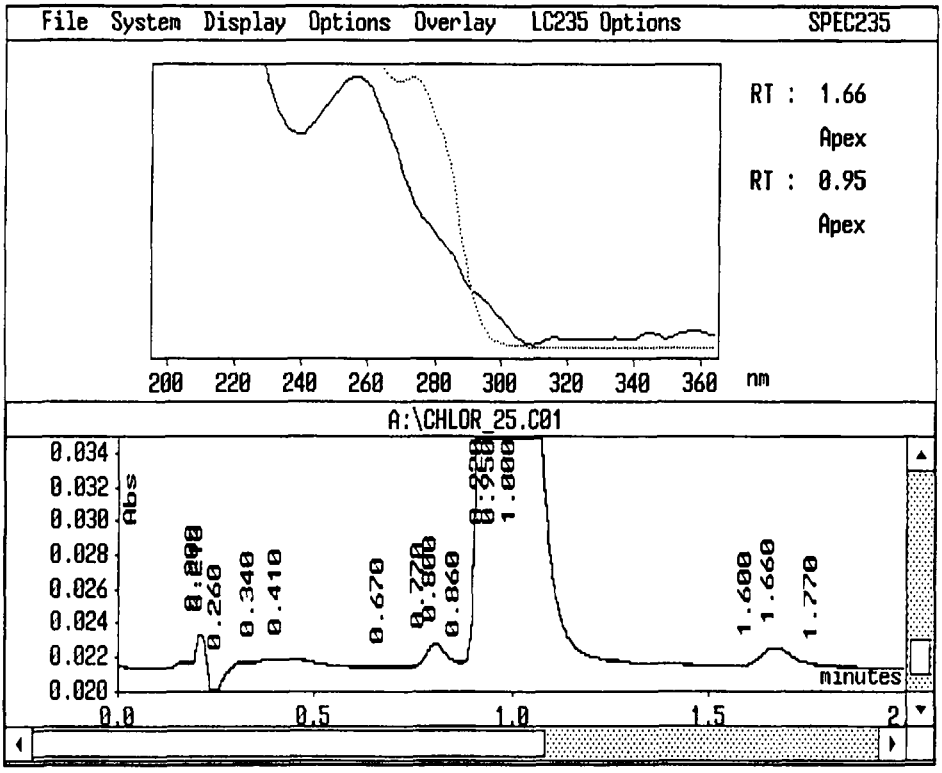

FIGURE 3. Screen page showing a high-sensitivity chromatogram of a chlorthalidone bulk sample (at 0.005 AUFS @ 235 nm). The upper diagram shows the normalized UV spectrum of CCA (solid line, max at 256 nm) and chlorthalidone (dotted line, max at 274 nm).

same chromatogram (shown in the lower trace of Figure 2) but at high sensitivity (0.005 AUFS). The chromatogram reveals the main chlorthalidone peak with two trace impurities. The peak at retention time 1.6 min was identified as CCA by its retention time and its UV spectrum. The spectrum (solid line) represents only 4 ng of CCA. Note that the UV spectrum is distinctively different than that of chlorthalidone (dotted line), indicating

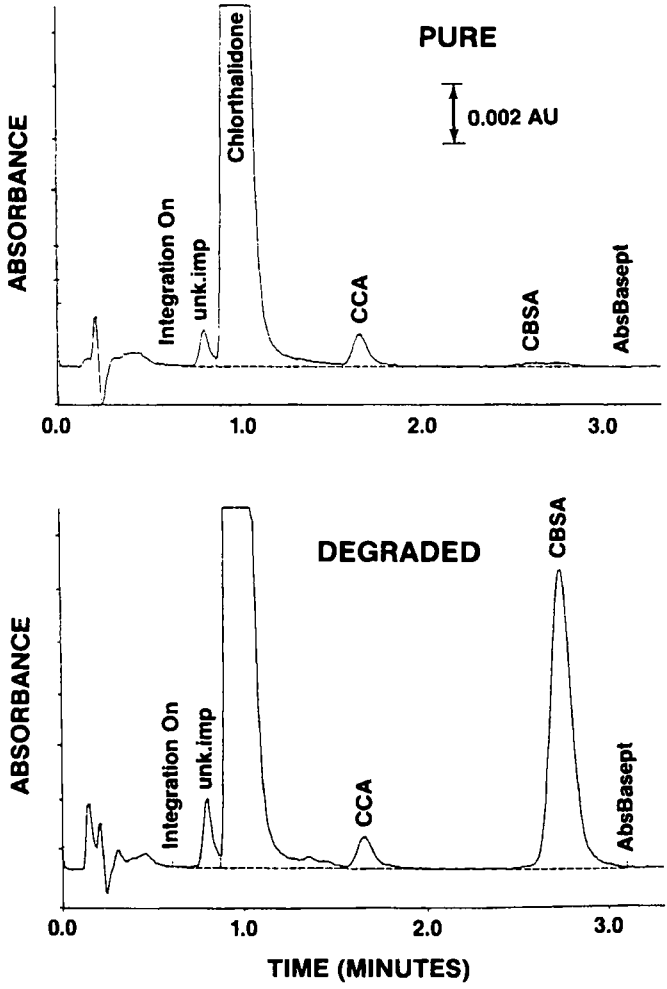

FIGURE 4. Comparative high sensitivity chromatogram of pure and degraded chlorthalidone showing the integration baseline and data handling time events. Integration baseline is also drawn under each peak. The judicious use of data handling time events is paramount to the accurate determination of the area of small peaks.

PURE CHLORTHALIDONE

NORMALIZATION (AREA)

RT	Area	BC	ExpRT	RF	Area %	Name
0.800	25855	T	0.800	3.18000e-8	0.2236	unk.imp.
0.960	13095406	T	0.950	2.78691e-8	99.2620	chlorthalidone
1.664	45607	V	1.660	3.18319e-8	0.3949	CCA
2.629	15806		2.630	2.78000e-8	0.1195	CBSA

DEGRADED CHLORTHALIDONE

NORMALIZATION (AREA)

RT	Area	BC	ExpRT	RF	Area %	Name
0.797	47701	T	0.800	3.18000e-8	0.4270	unk.imp.
0.957	12169496	T	0.950	2.78691e-8	95.4788	chlorthalidone
1.659	47970	V	1.660	3.18319e-8	0.4299	CCA
2.744	468204		2.630	2.78000e-8	3.6643	CBSA

FIGURE 5. Normalization area reports for the pure and degraded chlorthalidone samples shown in Figure 4.

the different chromophoric properties of the two structures (see Figure 1). Similarly, the unknown impurity eluting at 0.8 minutes was found to have a UV spectrum almost identical to CCA thus possibly indicating another hydrolysis product of chlorthalidone.

Figures 4 - 6 show the comparative chromatograms, reports and absorbance profile maps of bulk samples of pure and degraded chlorthalidone. The degraded sample was obtained by leaving the pure sample in the mobile phase for one day at room temperature. Note that the chromatogram, report and profile map clearly indicate higher levels of impurities in the degraded sample. The profile map offers a global representation of the unknown sample and facilitates the selection of the optimum monitoring wavelength. These examples illustrate the utility of DADs for the methods development of impurity assays.

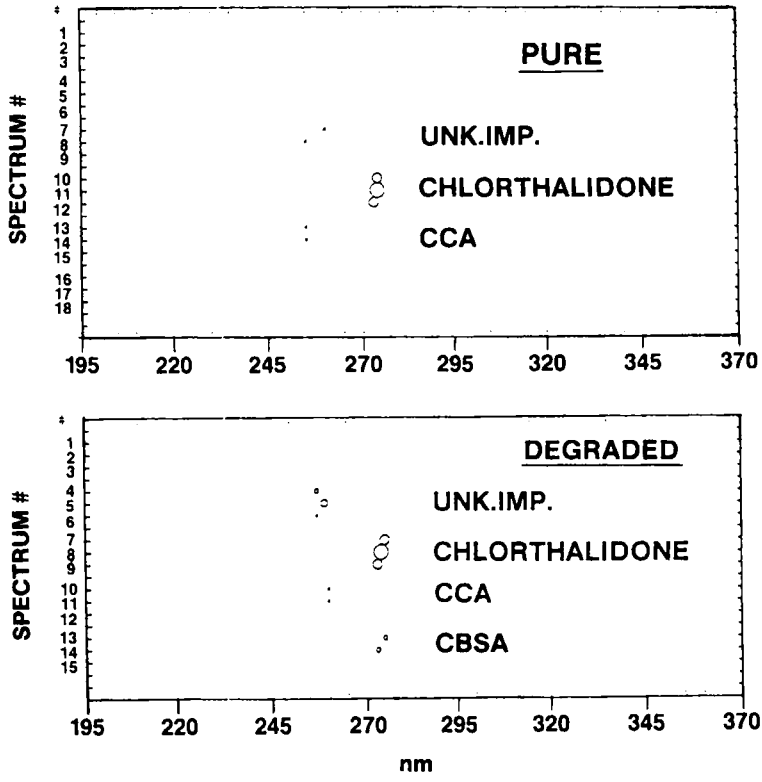

FIGURE 6. Comparative Absorbance Profile Maps of the pure and degraded chlorthalidone samples. These maps yield a quick overall profile of the samples.

Method Validation

Method validation and system suitability test. Method validation is the process to establish that performance characteristics of the analytical method are suitable for the intended applications. Performance characteristics are expressed in terms of analytical parameters such as those specified in Table 1. While validation is performed once during development on the entire analytical method (including sample preparation),

system suitability tests are performed on a given system (e.g., the particular LC in the lab) periodically to determine its adequacy or effectiveness. System suitability tests are defined in each compendial monograph and usually include precision, resolution and peak quality measurements. For instance, the USP system suitability criteria for chlorthalidone include:

- Repeatability of peak area from five replicate injections should be <2% RSD.
- Resolution between chlorthalidone and CCA should be > 1.5.
- Tailing factor of chlorthalidone should be < 2.

Other compendial monographs might include system suitability criteria such as column plate count, relative retention, or peak capacity factors (k').

Precision. Precision of an analytical method is the repeatability of the results in a series of experiments run during a single session with identical reagents and equipment by a single operator (6). Method precision is usually expressed as the relative standard deviation of the resulting values of the analyte concentrations. Method precision is dependent on the precision of the sample preparation and analysis procedure. Note that "reproducibility" is often defined as the agreement in the results of identical experiments made by the same operator on two different occasions or run in two different laboratories. While precision and repeatability are considered equivalent (same system, same operator on the same day), method reproducibility, in a narrow interpretation of the word, is also a consideration for "ruggedness" in this context (14).

LC system precision. Precision in retention time and peak area is a major criterion of the LC system suitability test. Precision data on a chlorthalidone tablet sample are shown in Table 3.

TABLE 3

Repeatability of Retention Time and Peak Area

Run No.	Chlorthalidone t_R	Chlorthalidone Peak Area	CCA (impurity) t_R	CCA (impurity) Peak Area
1	0.950	6087592	1.656	17242
2	0.957	6056092	1.658	17702
3	0.969	6051166	1.668	17231
4	0.958	6039786	1.662	17024
5	0.967	6098440	1.679	17951
6	0.970	6040052	1.683	17902
Mean	0.961	6062188	1.6676	17508
% RSD	0.083	0.41	0.067	2.4

NOTE: Injection volume was 10-μL by the ISS-100 autosampler under the variable injection mode.
Precision calculated by OMEGA-2 Peak Summary program.

Retention time precision. Retention time precision is important because retention time is the primary means for peak identification. It is also an important diagnostic for checking the performance of the LC pump and column.

Flow and temperature effects. Retention time precision for premixed solvents is controlled by the flow precision of the pump and temperature fluctuations of the column (14) as shown in equation 1.

$$\frac{\delta t_R}{t_R} = \left[\left(\frac{\delta F}{F} \right)^2 + \left(\frac{\delta \mu \delta T}{RT^2} \right)^2 \right]^{\frac{1}{2}} \quad \text{[Equation 1]}$$

where,

t_R = retention time
F = flow rate
T = temperature
$\Delta\mu$ = change in chemical potential
R = gas constant

According to one survey (14), the typical flow precision of an LC pump averages about 0.3%. However, many modern LC pumps are capable of much higher precision (i.e., 0.1%) (7, 15). While pump precision affects the reproducibility of retention time, the contribution from column temperature fluctuation is the more dominant cause for retention time drift. Since normal laboratory temperatures can vary as much as +/- 3 ^{o}C, retention time variation could be as great as +/- 1.5% (calculated from equation 1). Thus, for precison assays a column oven must be used.

Mobile phase composition. The use of a premixed solvent for isocratic analysis in liquid chromatography eliminates variability of the mobile phase composition which must be anticipated if the mobile phase mixture is pumped from two or more reservoirs. While many LC pumps can blend solvents accurately for methods development situations, since solute capacity factor varies in an exponential manner with changes in the mobile phase, preblended solvents should be used once an isocratic assay is developed (14). Continuous helium degassing also tends to change the mobile phase composition (for premixed solvents) by stripping away the volatile components and causes long-term retention time drift (15). A helium pressurization system is preferred for maintaining compositional integrity of the mobile phase.

Peak area precision. Factors affecting precision in peak area are listed in Table 3. The injection precision is the dominant factor controlling the repeatability of peak areas of all sample components. In contrast, noise and peak integration parameters become significant for measuring peak areas of low concentration components.

Injection precision. Use of autosamplers is widespread in the pharmaceutical laboratory. It is the best means for obtaining reproducible injections. Most modern autosamplers allow the selection of two operation modes: variable (or partial) and complete loop fill. Under the partial loop fill mode, precision of 0.4% - 1% RSD for injection volumes over 10 μL, is typical. Repeatability, however, usually deteriorates at lower volumes (<5 μL). For instance, assuming a precision of +/-0.05 μL for the loading syringe in an autosampler, the resulting precision will be 1% for a 10-μL injection but 5% for a 2-μL injection. The use of a complete loop fill mode improves precision to 0.3-0.5% levels, though a larger sample volume (4 times the loop size) is required to flush the sample loop completely.

Flow and temperature effects. Flow precision is a direct variable for peak area because absorbance detectors are concentration sensitive (16). Its effect, however, is often minor for modern pulse free pumps (0.1-0.2%). Column temperature affects retention time, k', and peak height, but has negligible effect on peak area (14).

Noise Effects. Peak area precision often degrades significantly on minor peaks due to noise contributions as described by equation 2 (14).

$$\frac{\delta A}{A} = \frac{4.}{\sqrt{2\pi}} \left(\frac{S}{N}\right)^{-1} \frac{W^{3/2}}{n} \qquad \text{[Equation 2]}$$

where, A = peak area
S/N = signal to noise ratio (SNR)
w = peak width
n = number of sampling points in the peak

This noise effect is evident in the precision data presented in Table IV where significantly higher RSD values (2.24%) were observed for the impurity peak CCA (0.3 area %) vs an RSD of 0.41% for the major peak (chlorthalidone 99.7%). The signal/noise ratio (SNR) of the CCA peak as measured from Figure 7 was found to be 20. Equation 2 indicates that precision is enhanced by increasing the SNR, decreasing peak width and using higher sampling rates. The optimum number of data points in a peak should be in the range of 20. Means for enhancing SNR are discussed further in the next subsection on detection limits.

Peak integration parameters. Today, the use of electronic integrators or data workstations for peak area measurement is universal. While peak integration algorithms are much improved over the years, and most devices can measure peak areas accurately, the precise measurement of small peaks is contingent on the proper selection of integration parameters (such as the peak start and peak termination point). Figure 7 illustrates some of the features of a modern data system (e.g., display of a magnified portion of the chromatogram and the integration baseline, etc.) which are important for the accurate integration of small peaks.

Accuracy. The accuracy is the closeness of test results obtained by the method to the true value. Accuracy is a measure of the exactness of the total analytical procedure, which is critically dependent on sampling, preparation, calibration and analysis procedures (6). Accuracy is usually expressed as percent recovery obtained by the assay of known added amounts of analyte in the sample matrix. Detailed discussions on accuracy can be found in most textbooks on quantitative analysis (17).

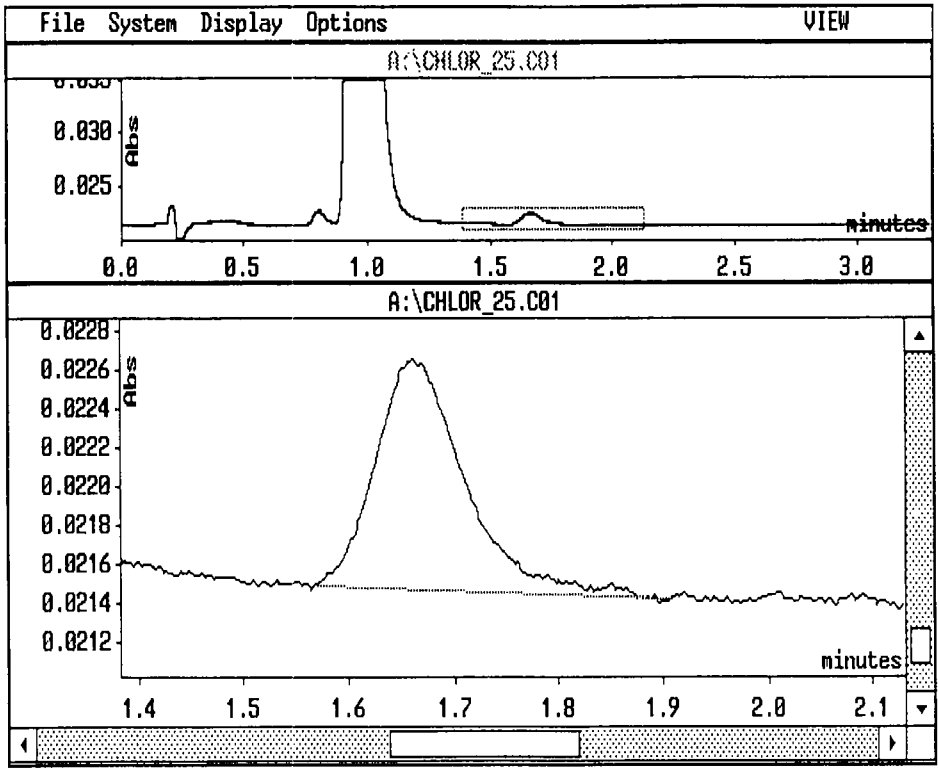

FIGURE 7. Screen page showing the chromatogram of a bulk chlorthalidone sample and a magnified portion containing the CCA peak.

Accurate results stem from correct selection of analysis method, error-free calibration, correct integration of peaks, system precision, separation resolution, linearity and the elimination of interferences and other systematic errors. A good understanding of the basic principles of analytical errors and their propagation is important for improving accuracy and precision.

TABLE 4

Factors Affecting Limits of Detection and Quantitation (For UV/Vis Detectors Only)

Signal	Monitoring wavelength
	Solute molar absorptivity
	Injection volume and mass
	Column efficiency
	Peak shape
	Solute retention
	Flow cell pathlength
Noise	Detector noise and drift
	Detector response time
	Mobile phase transparency
	Pump pulsation

Limit of Detection and Quantitation. The limit of detection is the lowest concentration of analyte in a sample that can be detected. Generally, a signal-to-noise ratio (SNR) of 2:1 or 3:1 is accepted. In contrast, the limit of quantitation is the lowest concentration of analyte in a sample that can be determined accurately. Typically, a SNR of 10 is the criterion (6). Figure 7 illustrates how these limits are obtained. Since 3 ng of CCA was injected and the SNR was estimated to be 20, the limits of detection and quantitation were found to be 0.3 ng and 1.5 ng, respectively for CCA.

Factors affecting the limits of detection are listed in Table 4. Since both the limits of detection and quantitation depend on SNR, they can be improved by enhancing analyte signals and reducing detector noise. Signal is enhanced by increasing peak height. This can be achieved in many ways: by selecting the optimum monitoring wavelength, by increasing injection volume or mass, and by increasing the peak sharpness with high efficiency

columns and optimized mobile phase. For absorbance detectors, longer pathlengths in the flow cell enhances sensitivity though often at the detriment of extracolumn dispersion. Noise can be reduced by using high sensitivity detectors with low noise and drift characteristics, slower detector response time, mobile phases with low absorbance and pumps with low pulsation. In addition, the selection of optimum peak integration parameters is an absolute prerequisite for precise quantitation of low level impurities (see previous section).

Note that conflicts sometimes occur when optimizing sensitivity and compromises must be made in order to balance sensitivity with other performance parameters. For instance, lowering solute k' increases peak height but at the expense of resolution. Also, the use of triethylamine (TEA) in the mobile phase often improves the peak shapes of basic drugs (enhances signals and peak shapes), nevertheless its absorbance at low UV also reduces sensitivity (increases noise). For the quantitation of trace impurities (<0.1%), the use of a special quantitation technique such as high-low injection (18) should be explored.

Selectivity. Selectivity or specificity is the ability of the analytical method to measure accurately and specifically the analyte in the presence of other components in the sample matrix (6). Selectivity in LC analysis is dependent on a number of operating variables such as the chromatographic modes, the column and mobile phase used, and the detection parameters.

During the initial phase of drug discovery and pharmaceutical development, the new drug candidate and its

impurities are chemically and toxicologically characterized. Major impurities and degradants should preferably be isolated for future reference. For the development of stability indicating assays, the analyst must establish method selectivity by demonstrating that the drug molecule is adequately resolved from all known degradation products, synthetic precursors/byproducts, process impurities and excipients (2,4). At this stage, other modes of LC such as thin layer chromatography (TLC) and normal phase LC including gradient elution, can be used (4).

Higher column efficiency enhances selectivity by increasing peak resolution. Reducing solvent strength often increases peak resolution but at the expense of analysis time. However, selecting alternate bonded phases (C8, cyano, amino), mobile phase modifiers (THF, ACN, pH, buffer, triethylamine) or detection mode (electrochemical, fluorescence) is often the preferred route. As described earlier, DADs offer another convenient way to establish peak purity and therefore selectivity.

Linearity and range. The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentrations of analyte in samples within a given range. The range is defined as the interval between the upper and lower levels of analyte that have been demonstrated with precision, accuracy and linearity (6).

During method validation, the linearity of detector response vs concentration of analyte injected must be checked. For absorbance detectors, deviations from Beer's Law are often encountered due to fluorescence, scattering and other chemical or

TABLE 5

Factors Influencing LC Method Ruggedness

o LC System	System reliability (7) Detector stability and wavelength resettability (8)
o Column:	Stability of stationary phase Column life (21) Batch-to-batch reproducibility Use of guard and precolumns (21)
o Mobile Phase	Rugged solvent conditions (22) Reproducibility of mobile phase composition
o Data Handling	Rugged integration algorithm (9)

physical effects (16). The linear range of a UV detector is primarily limited by its noise characteristics at the low end and by stray light characeristics at the high absorbance range (19). The linearity range of most absorbance detectors including DAD's is usually greater than 10^4 (16). The linear dynamic range of interfaces (analog/digital converters) for data stations is typically 10^6 to 10^7 (9). These ranges are more than adequate for most USP assays which generally call for linearity of 50% - 150% of the label claim (6).

Many integrators offer automatic calculation of response factors and method calibration from stored files. In addition, computer-based systems often facilitate the validation of linearity by allowing automatic multilevel calibration within a queue and by the plotting of calibration curves (9). Others also allow the implementation of linear, quadratic or cubic calibration with automatic calculation of intercepts and correlation coefficients.

Ruggedness. The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions (e.g., different labs, analysts, instruments, different columns and different days)(6). Table 5 lists the factors influencing LC method ruggedness. For continued control of the drug substances and formulated products over many years, ruggedness of the assay method is critical. Discussion of these factors can be found in the references indicated in Table 5.

Future Trends

In the last decade the pharmaceutical industry has seen rapid changes. Formulations are becoming more sophisticated. Lower doses are formulated with newer and more powerful drugs. The simple rapid-dissolving oral tablets are frequently replaced with enteric coated tablets, sustained release capsules and other novel delivery systems. The biotechnology revolution has generated its first wave of recombinant pharmaceutical products which requires more stringent quality assurance procedures (23). On the other hand, there is also increasing pressure to control costs (especially for generic drugs) to remain price competitive in the market place.

All these have far-reaching implications in the quality control of drug products. Since thousands of tests are performed just for methods development and validation alone, the use of Fast-LC will have a dramatic impact on laboratory productivity (20). Laboratory automation equipment, such as, autosamplers, robotics and more sophiscated data systems are increasingly utilized. Diode array detectors are used to furnish spectral data to augment chromatographic data. Chiral purity of drugs is

under intensive scrutiny by the scientific community and regulatory agencies, and chiral separation might become an assay requirement for many drugs in the near future (2).

CONCLUSION

This paper demonstrates the effective use of high sensitivity diode array detection and modern chromatographic software for the development and validation of high quality analytical methods applicable to pharmaceuticals.

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