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SELECTION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS IN PHARMACEUTICAL ANALYSIS

III[a]. METHOD VALIDATION

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SUMMARY

The most important steps in the validation of high-performance liquid chromatographic (HPLC) methods are discussed. The establishment of system suitability data and the assessment of peak purity are demonstrated on the example of bisquaternary amino steroids. For the recognition of incomplete resolution of adjacent peak pairs, the absorbance-ratio method in which the ratio of absorbances at two preselected wavelengths are plotted as a function of time in combination with the separation of sample components subjected to various chemical and physico-chemical treatments (stress conditions) is applied. The separation power and performance of the HPLC systems are characterized by the system resolution (SR) and system selectivity (SS). The special demands of stability-indicating methods are summarized.

INTRODUCTION

The main aim of pharmaceutical analysis is to obtain necessary qualitative and quantitative information about the sample to be tested. Since the quantitative analysis includes the total analytical procedure from the sample treatment to the evaluation of the analytical results, each step of the procedure can be separately evaluated to determine the weakest step that may influence the analytical results. The overall validation of a high-performance liquid chromatographic (HPLC) method can be considered as the sum of different validation steps to be included in the analytical process (both chromatographic and pre-chromatographic investigations).

In general, method validation involves the performance and interpretation of a series of experiments designed to reveal the most important characteristic of an HPLC method. Previous parts of our paper[1,2] dealt with the optimization of the mobile phase in reversed-phase and normal-phase systems. In this part, different aspects of the validation of previously optimized HPLC methods will be discussed. The principles used in the authors' laboratory will be demonstrated on the example of the

[a] For Part II, see ref. 2.

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TABLE I
COMPOUNDS INVESTIGATED AND PEAK NUMBERS
Structures are shown in Fig. 6.

Compound	*Peak No.*
2β,16β-Bis(4'-methyl-1'-piperazino)-3α,17β-diacetoxy-5α-androstane	1
2β-(4'-Methyl-1'-piperazino)-16β-(4'-dimethyl-1'-piperazino)-3α,17β-diacetoxy-5α-androstane bromide	2
2β-(4'-Dimethyl-1'-piperazino)-16β-(4'-methyl-1'-piperazino)-3α,17β-diacetoxy-5α-androstane bromide	3
2β-(4'-Dimethyl-1'-piperazino)-16β-(4'-dimethyl-1'-piperazin-2',3'-ene)-3α,17β-diacetoxy-5α-androstane dibromide	4
2β-(4'-Dimethyl-1'-piperazin-2',3'-ene)-16β-(4'-dimethyl-1'-piperazino)-3α,17β-diacetoxy-5α-androstane dibromide	4
2β,16β-Bis-(4'-dimethyl-1'-piperazino)-3α,17β-diacetoxy-5α-androstane dibromide (Pipecuronium bromide)	5
2β,16β-Bis-(4'-dimethyl-1'-piperazino)-3α-hydroxy-17β-acetoxy-5α-androstane dibromide	6
2β,16β-Bis-(4'-dimethyl-1'-piperazino)-3α-acetoxy-17β-hydroxy-4α-androstane dibromide	7
2β,16β-Bis-(4'-dimethyl-1'-piperazino)-3α,17β-dihydroxy-5α-androstane dibromide	8

separation of pipecuronium bromide and related substances. Optimization of the separation system has been published[3,4].

EXPERIMENTAL

The same instrumentation (HP 1090A) as described in Part I[1] was used. Separations were performed on a LiChrosorb Si 60 (5 μm) column (250 × 4.6 mm I.D.) (Chrompack, Middelburg, The Netherlands). The eluent was methanol–acetonitrile–concentrated ammonia solution (43:43:14) containing 100 m*M* each of ammonium carbonate and ammonium chloride. The flow-rate was 1 ml/min and the steroids were detected at 213 and 225 nm.

The compounds to be tested were prepared at the Chemical Works of Gedeon Richter (Budapest, Hungary) and their quality was checked by HPLC prior to use. The compounds are listed in Table I and their structures can be seen in Fig. 6.

RESULTS AND DISCUSSION

The validation of every HPLC procedure involves at least four distinct steps: validation of sample pre-treatment and derivatization; chromatographic separation; elaboration of system suitability data; and peak purity determination. The analytical method itself using HPLC can be separated into the following five distinct parts: (i) sample pretreatment and preparation, including pre-column derivatization if necessary; (ii) introduction of the sample into the chromatographic system; (iii) chromatographic separation; (iv) detection and amplification of detector signals; and (v) transformation of detector signals into numerical data. The last four parts are termed instrumental components, because the scale of errors depends on the degree of instrumentation, and therefore validation of sample pre-treatment and preparation can be distinguished and discussed.

Validation for sample pretreatment and derivatization

Several books and reviews have dealt with the problems of sample preparation and derivatization and their contribution to the errors made during the analysis[5–9]. The errors made during sample preparation can be classified into five groups: sampling error; extraction of sample from the matrices; sample clean-up and enrichment; stability of the sample during the preparation of the test solution and during its storage; and errors caused by derivatization. Error contributions in sample pretreatment have been discussed by Snyder and Van der Wal[5], who developed a comprehensive theory of the various contributions to assay imprecision, providing specific conclusions and recommendations for significant improvements in precision.

Here, only three aspects closely connected with the practical approach of method validation are considered: recovery; stability of sample components during sample preparation and storage; and determination of the accuracy and precision of the sample pretreatment procedure.

Recovery of sample components. Recovery is a measure of the efficiency of the extraction of the analyte from the sample matrix. With respect to the sample type being analysed, two different types of samples can be distinguished. First, a mixture of a known number of essentially known compounds (*e.g.*, formulated pharmaceuticals) is analysed. Recovery can be determined by analysing a spiked placebo containing all ingredients except the active substance. Known concentrations of standard prepared from the active substance in increasing amounts (50, 75, 100, 125 and 150% of the labelled amount for dosage forms) are added to the placebo. The procedure is carried out in a manner identical (the sample is pulverized, milled, dried, homogenized, extracted and analysed) with that for the real sample. With a knowledge of the added and measured amounts , the recovery of the sample preparation can be calculated. In the second type, a mixture of a known number of components with a number of unknown (background) compounds is analysed. Here, a standard recovery graph is produced by adding increasing amounts of standard to the untreated sample.

Stability of sample components during sample preparation and storage. Many solutes readily decompose prior to chromatographic investigations during the preparation of sample solutions (extraction, clean-up, phase transfer, etc.) and also in ready-made sample solutions. To avoid this problem, several possibilities exist if we consider the possible reason(s) for the undesirable decomposition of sample components.

To determine the stability of the samples being analysed in a sample solution, the term of "system stability" (St_s) is defined. It is a measure of bias in the assay results within a preselected time interval (*e.g.*, every hour up to 4–6 h) using single solution. System stability should be determined by replicate analyses of the same sample solution and the results are evaluated for major and/or minor components.

System stablity is considered to be appropriate if the relative standard deviation calculated on the assay results obtained in different time intervals does not exceed more than 20% of the corresponding value of the system precision (discussed later). If the value is higher on plotting the assay results as a function of time, the maximum duration of the usability of the sample solution can be calculated.

Determination of precision of sample preparation. Because the method validation data for the overall analytical procedure are calculated from the detector responses after chromatographic separation, the precision data of sample pretreatment can be

separated from those of chromatographic separation. In the authors' laboratory the following procedure is used for this purpose. From seven independent weighings, seven sample solutions are prepared using an identical sample treatment in each instance. From each individual sample solution a single injection is performed. From the data, the precision characteristic of the overall analytical procedure is obtained (method precision, σ_m). From a single solution, seven determinations are made; the precision characteristic of the chromatographic procedure can be determined (system precision, σ_s). Finally the precision of sample preparation (σ_p) can be calculated from the first two precision data:

$$\sigma_p = \sqrt{\sigma_m^2 - \sigma_s^2} \quad (1)$$

Validation of chromatographic separation

Current concepts for an HPLC method validation procedure have been reported[10] and discussed in detail recently[11,12]. According to these guidelines,

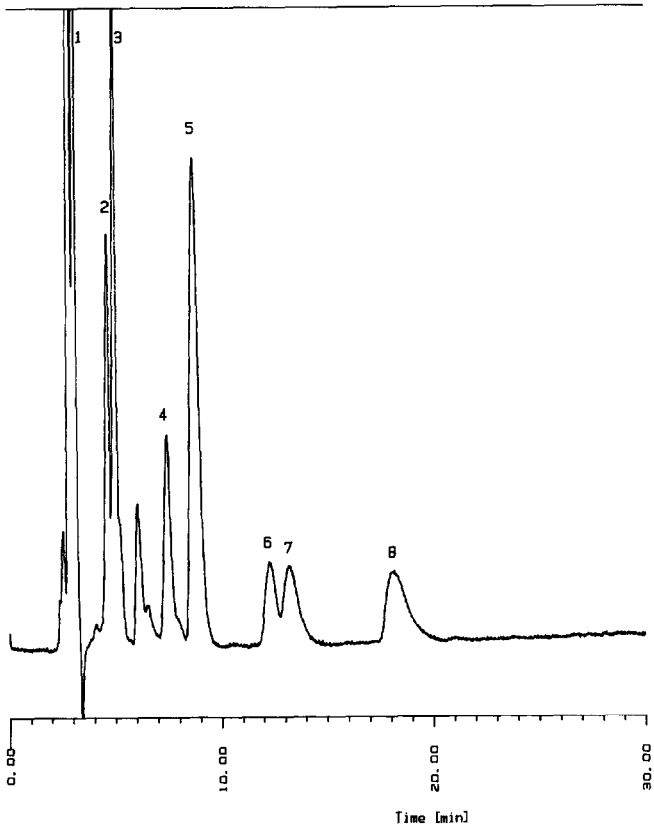

Fig. 1. Separation of pipecuronium bromide and its possible impurities. Conditions: column, LiChrosorb Si 60 (5 μm) (250 × 4.6 mm I.D.); eluent, methanol–acetonitrile–concentrated ammonia solution (43:43:14) containing 100 m*M* each of ammonium chloride and ammonium carbonate; flow-rate, 1 ml/min; detection at 213 nm. For peak numbers see Table I.

demonstration of the following data elements are required for HPLC method validation: accuracy, precision, limit of quantitation, selectivity, range, linearity and ruggedness. According to our classification, the validation data can be divided into four major groups:

(a) Data elements which can be statistically evaluated (their definitions and determinations can be found elsewhere[10–12]), such as accuracy, precision, reproducibility (repeatability), day-to-day reproducibility, inter-laboratory reproducibility (ruggedness), detector linearity and range (these data elements are not discussed here).

(b) System suitability data containing measures of the resolving power of the HPLC system and comprising criteria established for acceptance or rejection of any analytical results including additional data to characterize the performance of the separation system, such as column loadability, depending on the size of column hardware and type of stationary phase filled into the column.

(c) Peak purity test to verify the homogeneity of a chromatographic peak.

(d) Additional data such as system resolution (SR) and system selectivity (SS), which directly express the quality of the separation, proportional to the performance (selectivity of the separation) and power (efficiency of the separation) of an HPLC

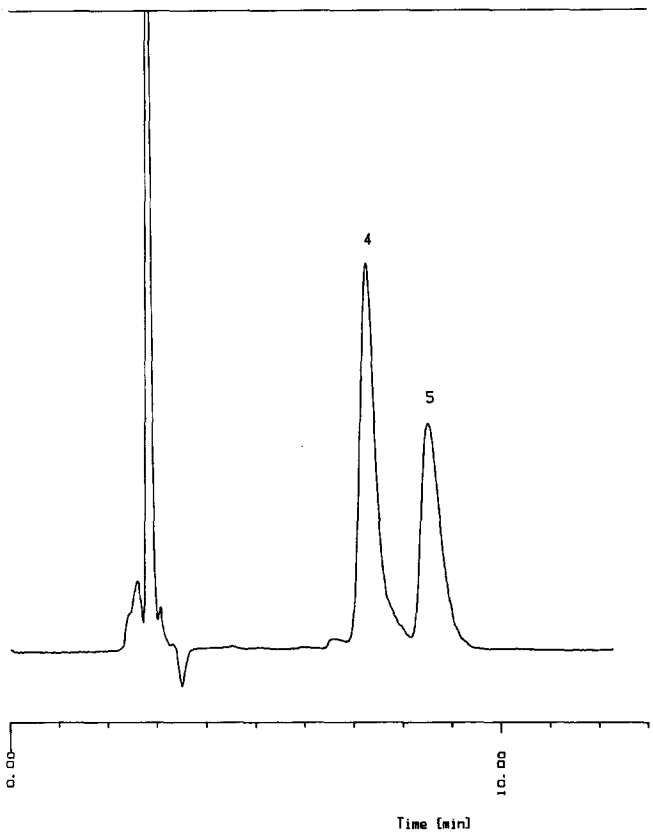

Fig. 2. Chromatogram used for the determination of system suitability. Conditions as in Fig. 1. For peak numbers see Table I.

system, and characterize the applicability of an HPLC method for solving a particular analytical problem.

System suitability data. The data elements belonging to the term system suitability can be controlled each day for a preselected sample (mainly for standard solutions used for quantitation) prior to the use of method for routine analysis to ensure that the system is performing up to specified standards. These are illustrated on the example of the separation and determination of pipecuronium bromide and its impurities listed in Table I. The standard chromatogram and the chromatogram used for the system suitability test are shown in Figs. 1 and 2. System suitability data are collected in Table II. The following points can be considered.

(a) Minimum required resolution measured between two adjacent peak pairs: as can be seen in Table II, the poorest separated pair of peaks are 2 and 3 (two monoquaternary derivatives), with an $R_{s,min}$ value of 1.13. However, these are impurities present at low concentrations. The separation between peaks 4 and 5 (characterized by R_{sb}; see Part I[1]) is more important, as compound 4 is the main degradation product. Therefore, the minimum value of the required resolution (R_s) is determined for these two components (4 and 5).

(b) The approximate capacity ratio is determined for pipecuronium bromide to estimate the possible retentions of the other components.

(c) The maximum allowable value of the peak asymmetry is determined for the peak of pipecuronium bromide and serves to control the conditions of the separation column.

(d) The approximate value of the peak height is also determined for the peak of pipecuronium bromide using fixed detection and amplification parameters to control the detection conditions.

(e) The maximum value of the column loadability can be characterized by the amount of sample resulting in not more than a 20% decrease in the theoretical plate number[13]. It is determined for the main component (pipecuronium bromide) and can be considered when larger amounts of sample are introduced in order to improve an unsatisfactory detectability of trace components.

(f) The limit of quantitation (lowest detectable quantity, LDQ) is a parameter of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in pharmaceuticals. It can be expressed as the lowest concentration (quantity) of an analyte in the sample which can be determined under the prescribed experimental conditions with acceptable accuracy and precision. It should be determined for the major component and for minor compounds differing significantly in structure (*e.g.*, for peaks 4 and 5).

(g) The maximum acceptable value for the relative standard deviation is determined for pipecuronium bromide by multiple injections of a standard solution.

(h) Linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with various concentrations of analyte. The slope of the regression line and its variance provide a mathematical measure of linearity; the intercept on the ordinate is a measure of the potential assay bias.

Peak purity. One of the most important parts of the method validation procedure is to confirm the purity of a chromatographic peak. Several published techniques[14–23] are available for examining the purity of a peak profile. One of the

TABLE II
METHOD VALIDATION DATA FOR THE DETERMINATION OF PIPECURONIUM BROMIDE
Conditions as in Fig. 1.

Data	*Parameter*	*Value*	
System suitability data	Retention times:		
	For peak 4 (decomposition product)	7.3 min	
	For peak 5 (pipecuronium bromide)	8.5 min	
	Resolution for peaks 4 and 5	Min. 1.5	
	Relative standard deviation for peak 5	Max. 1.0%	
	Asymmetry factor (for peak 5)	Max. 2.0	
	Column loadability (for peak 5)	100 μg	
Method validation data	R_{sb} (for peaks 4 and 5)	1.74	
	R_{sa} (for peaks 5 and 6)	3.81	
	$R_{s,min}$ (for peaks 2 and 3)	1.13	
	D_{min} (for peaks 2 and 3)	0.072	
	SS	−0.013	
	SR	1.85	
	Asymmetry factor for peak 5	1.55	
	Lowest detectable quantity:		
	for peak 5 (pipecuronium bromide)	25 ng	
	for peak 4	4 ng	
	Relative standard deviation (for peak 5)	0.81%	
	Day-to-day reproducibility (for peak 5)	1.97%	
	r^2	0.999	
	F	999.9	
		Response ratio measured at 213 and 225 nm	
		Treated sample	*Spiked sample*
Peak purity	Stress conditions:		
	Standard	3.927	
	Reflected light, 14 days	3.999	3.999
	40°C, 80% relative humidity, 14 days	3.922	3.919
	105°C, 48 h	3.987	3.975
	UV light (254 nm), 24 h	3.935	3.891
	pH 2, RT[a], 24 h, 2% solution	3.866	3.850
	pH 12, RT[a], 30 min, 2% solution	3.699	3.712
	Relative standard deviation ($n=13$)	3.899 ± 2.51%	
	$D = (k_2 - k_1)/(k_2 + 1)$		

[a] Room temperature.

simplest methods is to plot the ratio of the absorbances at two (or more) preselected wavelengths as a function of time[20]; peak inhomogeneity is indicated by a discontinuity in the plot. The techniques, however, have a common problem, namely that peak inhomogeneity can be mostly recognized when the spectral properties of the overlapping compounds are sufficiently different and total overlap of two peaks does not occur.

To improve further the assessment of peak purity investigations, a combination of the method based on the measurement of absorbance ratios with time[20] with the

separation of samples subjected to appropriate stress conditions producing decomposition products may be applied in order to recognize peak overlapping. The length of time a substance is subjected to stress conditions depends on the rate of degradation. A degradation of 10–15% is considered to be adequate. (Stress conditions used for the peak purity investigation of pipecuronium bromide are listed in Table II.

The experiments start with the determination of the absorbance ratio(s) for a chromatographically pure (standard) compound at two (or more) preselected wavelengths with respect to time from the standard chromatograms. The chromatograms of treated samples without and with spiking with known concentrations of a standard are recorded at the selected wavelengths. The absorbance ratios (for the peak of pipecuronium bromide) are determined as a function of time.

The following possibilities may exist:

(a) When two peaks do not totally overlap and the difference in spectral properties is sufficient, peak inhomogeneity can be recognized from the discontinuity in the absorbance ratio(s) with time.

(b) When two peaks do not totally overlap, but the compounds possess similar spectral characteristics, existing differences in their decomposition rates can cause changes in the peak shape, resulting in different absorbance ratios for the treated, spiked and standard samples.

(c) When two peaks totally overlap, but the spectral properties of the two compounds are sufficiently different, peak inhomogeneity can be recogized from the apparent difference in the degree of degradation observed for real and reference pure samples owing to the different decomposition rates. The degree of degradation seems to be higher when the main component has a significantly lower absorbance at the selected wavelengths and a slower decomposition rate than the co-eluting compound. The reverse situation exists when the main component has a higher absorbance and a faster decomposition rate.

(d) The most problematic cases of possible peak inhomogenicity are as follows:

(i) peak inhomogeneity belongs to group (b), but no significant difference in decomposition rates exists between the co-eluting compounds;

(ii) peak inhomogeneity belongs to group (c), but the main component has a higher absorbance and slower decomposition rate than the co-eluting compound and, conversely, when the co-eluting compound has a higher absorbance and a slower decomposition rate;

(iii) two peaks are totally overlapped and the spectral properties of the co-eluting compounds are similar.

In this instance the peak inhomogeneity can be recognized only from the chromatograms obtained for the treated sample, assuming different decomposition products are formed from the co-eluting compounds under stress conditions resulting in alteration in the elution patterns for the treated standard and sample solutions.

To increase the possibility of the formation of different degradation products, stress conditions include heat, light and humidity treatments and decomposition due to the pH in aqueous solutions (or for solubility reasons in a mixture of water and organic solvents).

In our practical experience, when the peak absorbance ratios at two (or more) wavelengths determined for treated, spiked and non-treated samples are within acceptable limits ($\pm 5\%$), the chromatographic peak can be considered to be pure. A typical example (pipecuronium bromide) is shown in Fig. 3.

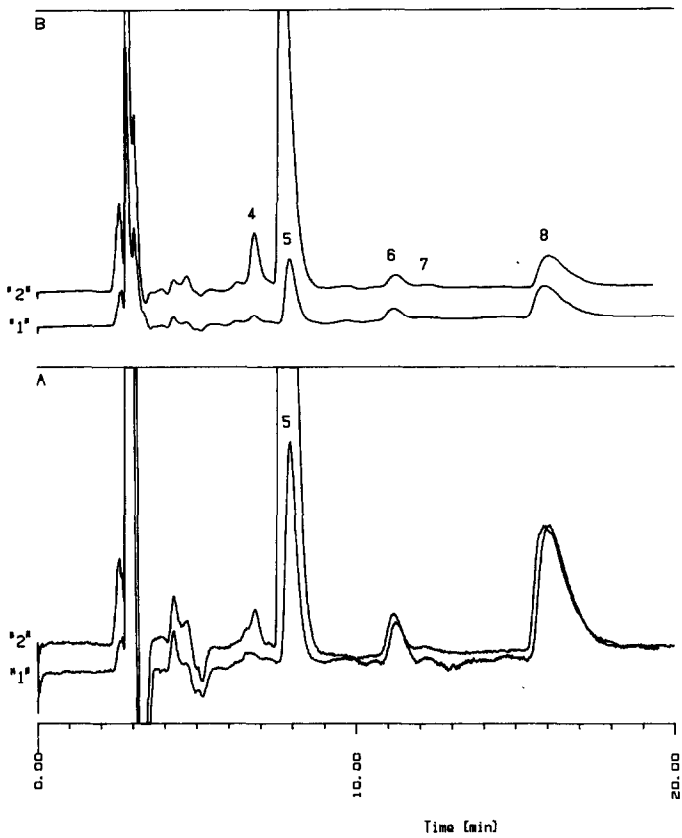

Fig. 3. Assessment of peak purity. (A) Detection at 213 nm; (B) detection at 225 nm. (1) Chromatogram of treated sample (2% aqueous solution at pH 12; sample allowed to stand at room temperature for 30 min). (2) chromatogram of treated sample spiked with standard pipecuronium bromide. Peak ratios (measured for pipecuronium bromide): 3.699 for treated sample and 3.712 for spiked sample. Conditions as in Fig. 1. For peak numbers see Table I.

It should be noted that the application of direct thermospray mass spectrometry to liquid chromatographic eluents is an extremely powerful method of validating peak purity in every instance considered above.

Method validation data for the HPLC determination of pipecuronium bromide are collected in Table II.

System selectivity (SS) and system resolution (SR). When further information may be necessary about the quality of the separations from the point of view of the difficulties created by the analytical problems to be solved, it may be obtainable from the data from previous experiments carried out to formulate criteria that directly express the quality of the separation and are proportional to the performance (system selectivity) and power (system resolution) of an HPLC system.

The separation power of any HPLC system depends mainly on three parameters: the resolutions achieved between the peak of the main component and preceding (R_{sb}) and following (R_{sa}) peaks, and the lowest value of the resolution ($R_{s,min}$) obtained for

any pair of peaks in the chromatogram. Values required for R_{sb} and R_{sa} are the functions of the relative concentrations of compounds in the sample (peak ratios of the adjacent peak pairs), and these are also dependent on the analytical problems to be solved (this will be discussed in Part IV). Here their recommended values are indicated by b (for R_{sb}) and a (for R_{sa}). If an HPLC system possesses these recommended resolution data, that system can be considered to be applicable for solving the analytical problem. The most advantageous value for both R_{sb}/b and R_{sa}/a is 1, as a lower value is not sufficient for a perfect separation and a higher value will increase the analysis time.

When all the peaks on the chromatograms are treated as being of equal importance, the minimal resolution measured for the worst separated peak pairs should also be considered. Based on the above-mentioned considerations, the system resolution (SR) can be expressed by the following equation:

$$\mathrm{SR} = \frac{R_{sb}}{b} \cdot \frac{R_{sa}}{a} (1 + R_{s,min}) \tag{1}$$

The first part of the SR equation is important when the compounds are present at different concentrations. The second part is important when compounds at similar concentrations can be identified. The maximum values for R_{sb}/b and R_{sa}/a are limited to unity to avoid false results being obtained by multiplication of a high value of one with a low value of another (in this instance SR is a function of $R_{s,min}$). When no peak elutes before or after the peak of the main component, the ratio is also unity.

System selectivity (SS) refers to the selectivity of the total separation system and can be expressed by the equation

$$\mathrm{SS} = \frac{D_a^z}{(1 - D_{min}^{z+1})^{1-z}} - \frac{D_a^v}{(1 - D_{min}^{v+1})^{1-v}} = Z - V \tag{2}$$

where z is the number of peak eluting before the peak of the main component; v is the number of peaks eluting after the peak of the main component;

$$D_a^z = \frac{\Sigma D^{z+1}}{z} = \text{average } D \text{ value calculated for peaks "}z\text{";}$$

$$D_a^v = \frac{\Sigma D^{v+1}}{v} = \text{average } D \text{ value calculated for peaks "}v\text{";}$$

$$D_{min}^{z+1} = \text{lowest value of } D \text{ for peaks "}z\text{";}$$

and

$$D_{min}^{v+1} = \text{lowest value of } D \text{ for peaks "}v\text{".}$$

The system selectivity depends on the values of Z and V and directly correlates with the separation for the peaks eluting before (z) and after (v) the main component. When the

value of Z is higher than V, it provides better separation conditions. Values of Z are higher (a) when more peaks elute before the peak of the main component than after it (z is higher than v); (b) the average normalized resolution (D_a) and/or the minimum value of the normalized resolution (D_{min}) calculated for the peaks eluting before the main component is higher than the corresponding value for the peaks eluting after the main component.

When no peak elutes before the main component ($z=0$), the system selectivity is equivalent to the value of V, and similarly SS has a positive value when no peak elutes after the main components ($v=0$, SS$=Z$). When only one peak elutes before or after the main components, the values of Z and V are equivalent to the corresponding values of the minimum normalized resolutions.

With respect to the system selectivity, it can be generally concluded that its value is dependent mainly on the elution order provided by the performance of the given separation system and has great importance when trace compounds are determined. A prefered separation system can be selected on the basis of the numerical value of SS (see Part IV).

Stability-indicating methods

The purpose of stability tests is to obtain adequate information that enables proposals to be made for the shelf-life of pharmaceutical products and to recommend storage conditions. Appropriate stability tests require the use of appropriate stability-indicating methods. Special demands placed on stability-indicating methods can be summarized as follows:

(a) The peak of the main component (drug substance) should not co-elute with any other peaks originating from its production (by-products) or formed by decomposition (degradation products); the method should be able to follow the decrease in active content during the period of the stability investigations (stability-indicating assay method).

(b) Desired resolutions between the peak of the main component and adjacent peak pairs (R_{sb} and R_{sa}) can be selected to be higher than in other instances (this will be discussed in more detail in Part IV), in order to identify possible degradation products similar in structure and chromatographic characteristics, and formed during various storage conditions at low concentrations (stability-indicating purity testing method).

(c) The optimum k' value for the main component is between 5 and 8 to achieve the necessary band spacing for the possible decomposition products with different chemical natures.

(d) The desired value of the precision of stability-indicating assay methods should not be more than $\pm 1.0\%$, in order that small decreases in active content can be accurately measured.

(e) The peak(s) of decomposition product(s) with different chemical natures should be separated from those of impurities present in the sample at the start of the investigations, as the results of assay and purity tests are evaluated together and can be corrected using the original impurity content.

(f) Peaks of secondary decomposition products (formed by degradation of by-products and/or decomposition products) can also be separated from other peaks.

To satisfy these requirements, good chromatographic resolution (suitable values of SR and SS) and well defined stress conditions (see Table II) can be established.

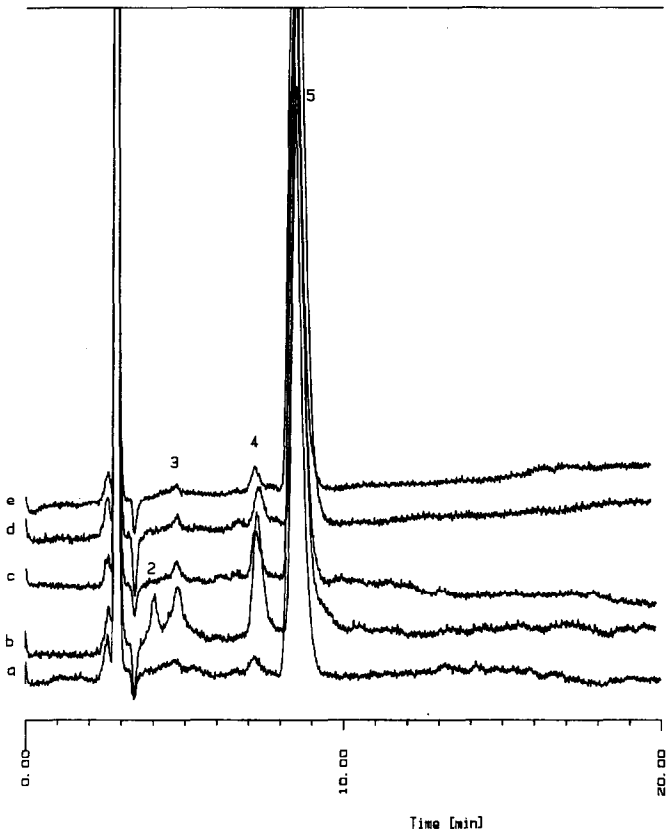

Fig. 4. Chromatograms of pipecuronium bromide subjected to various treatments in solid form. (a) untreated sample; (b) heated at 105°C for 48 h; (c) irradiated with reflected light for 14 days; (d) heated at 40°C and 80% relative humidity for 14 days; (e) irradiated with UV light (254 nm) for 24 h. Conditions as in Fig. 1. For peak numbers see Table I.

Chromatograms obtained for a pipecuronium bromide sample subjected to different stress conditions are shown in Fig. 4 (treatments made in solid form) and in Fig. 5 (treatments made in solution).

CONCLUSIONS

Based on the chromatograms obtained for pipecuronium bromide subjected to various treatments, the degradation pathway shown in Fig. 6 can be proposed.

Considering the most important characteristics of the validation of HPLC methods, it can be generally concluded that in addition to the well known criteria (accuracy, precision, linearity, range, sensitivity, ruggedness) published as guidelines[10], proof of the selectivity (specificity) of an HPLC method is the main interest. This may be accomplished by adding known compounds (impurities, degradation products) in small amounts to known amounts of drug substances or by subjecting samples to appropriate stress conditions such as those mentioned above in order to

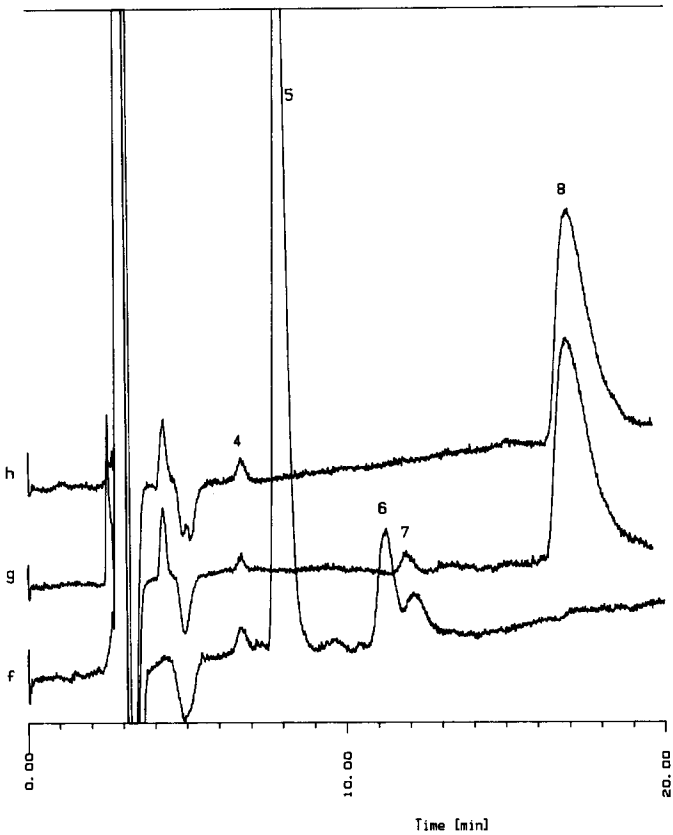

Fig. 5. Chromatograms of pipecuronium bromide subjected to various treatments in solution. (f) 2% aqueous solution at pH 2 and room temperature for 24 h; (g) same as (f) but at 100°C for 8 h; (h) 2% aqueous solution at pH 12 and 100°C for 8 h. Conditions as in Fig. 1. For peak numbers see Table I.

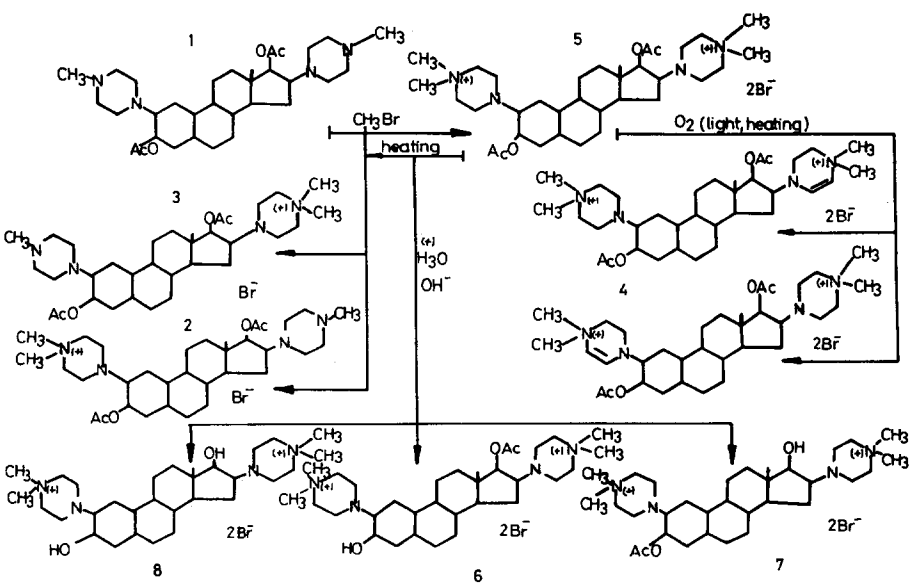

Fig. 6. Degradation pathway for pipecuronium bromide. Ac = acetyl.

generate "real" degradation products. Subsequently the purity of the peak of the main component should be determined adequately by absorbance ratio comparisons. Finally, the usability of the HPLC method for solving various analytical problems can be expressed in terms of SR and SS. The use of SS and SR as validation criteria can be recommended in pharmaceutical analysis.

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