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Qualitative Analysis by Derivative Spectrophotometry (II)

—Computer-assisted spectral analysis using derivative spectra and Root Mean of Squares of differences—

Man Ki Park, Jeong Hill Park and Jung Hwan Cho

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

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Abstract☐A computer program which can differentiate compounds whose absorbance spectra are very similar was developed. The program, [SPECMAN PLUS], written in Pascal provides automated spectral comparison techniques, utilizing the values of Root Mean of Squares (RMS) of differences. This comparison routine of the program can deal with spectra of compounds of different concentrations and different spectral recording resolutions. In addition, the program was designed applicable to any spectral data of digital form. The program was applied to the UV spectra of 13 penicillins and 5 cephalosporins, whose absorbance spectra are so similar. As a result, all compounds examined could be differentiated from each other.

Keywords☐Derivative Spectrophotometry, computer program, computer-assisted spectral analysis, root mean of squares (RMS) of differences.

Derivative spectra have been used with various purposes in qualitative and/or quantitative analysis[1-5]. One of the problems in this technique is the significant decrease of S/N ratio by the derivative operations. The various digital filters were introduced to solve this problem[6]. As a consequence, the higher-order derivative spectra are practical for various applications. With higher-order derivative spectra, the fine structures of the absorbance spectra can be revealed, and this can be used as a parameter for the similarity measure between the compounds of similar structures, whose absorbance spectra show similar patterns. This was discussed in a previous report by the authors[1]. The methods for the determination of similarity between two spectra of question have been discussed in many aspects. In the cases of IR spectra, the important parameters for the similarity measure were the peak location and the peak shape[7] and these were compared by various methods such as the match sum[8], the Hamming distance[9], and the Euclidean geometric distance[10]. Fourier transform technique was used for the similarity measure in IR[11-14] and UV/visible spectroscopy[15], but this technique took much more tedious time for the calculation of Fourier transform. Therefore, another reasonable parameter of practical value is needed as a similarity measure for the UV/visible spectra. For the practical application of the resolution enhancement feature of the derivative operation, a new computer program written in Pascal language was developed for IBM/PC/XT/AT compatible computers. The root mean of squares (RMS) of differences was employed in the program as a similarity measure.

EXPERIMENTAL METHODS

Instruments

UV spectrophotometer used for the experiment was Ultrospec 4050 of LKB Biochrome, England, and computer was IBM/PC/AT computer of IBM, U.S.A. The communication between the spectrophotometer and the computer was accomplished via RS-232c communication line. The program was written in Turbo Pascal of Borland International, U.S.A. All data used by the program were recorded on the Seagate hard disk system.

Materials

Amoxicillin trihydrate, ampicillin trihydrate, bacampicillin, carbenicillin, carindacillin, cyclacillin, dicloxacillin, floxacillin, hetacillin, metampicillin, penicillin G, penicillin V, piperacillin, cefazolin, cefotaxime, cephalexin, cephaloridine and cephradine were purchased from Sigma Co., U.S.A.

All compounds listed above were dissolved in the phosphate buffer (pH 7.4), respectively. Concentration used for the acquisition of reference spectrum was 20 μg/m*l* except bacampicillin and cyclacillin whose concentrations were 100 μg/m*l*.

Computer Program Procedures and Their Theoretical Backgrounds

A new computer program named [SPECMAN PLUS] provides 8 facilities for the spectral analysis.

[PLOT] procedure provides the auto-scaled spectra of % transmittance, absorbance and derivatives of 1st to 4th orders using digital data of absorbance spectrum of a certain compound registered to the file system of [SPECMAN PLUS]. % Transmittance data are calculated from absorbance values prior to graphics. Derivative spectra are calculated from absorbance values by convolution operation[16) using the convoluting integers prior to graphics.

[ADDSUB] procedure calculates addition spectra and/or subtraction (or difference) spectra from two spectra. In this calculation, user-defined factor can be incorporated.

[SYNSPEC] procedure provides synthetic spectra according to two models, the Gaussian distribution model and the Lorentzian distribution model. This procedure should be provided with some parameters for the spectrum synthesis such as the spectral range, the spectral recording resolution, the number of peaks, the locations of absorption maxima, the peak height and the full width at half maximum (fwhm).

[OVERLAY] procedure provides overlay spectra of compounds selected by the user. This procedure employed four kinds of graphic window types, which make it possible for the user to compare several spectra of the different compounds in a look.

[COMBINE] procedure provides graphics of 4 different spectra of one compound for the visual inspection of the change in pattern by the derivative operation.

[PEAK] procedure presents with automatic peak picking facility.

[VALUE] procedure presents with calculated values of % transmittance, absorbance and derivative at any wavelength in the range of spectral recording, regardless of the recording resolution.

[COMPARE] procedure is the most important one for the spectral analysis by derivative spectrophotometry. RMS difference of spectral data was used as a similarity measure. The value of RMS was calculated using the following equation.

$$\mathrm{RMS} = \frac{\sqrt{\Sigma(X_i^C \times f - x_j^R)^2}}{N}$$

where X_i^R is the spectral value at *i*th data point of reference spectrum, X_j^C is the spectral value at *j*th data point of comparing spectrum, *i*th and *j*th data points represent the same wavelength of two spectra of question, respectively, N is the number of spectral sampling points used for the comparison, and finally *f* is the factor which is introduced automatically to compensate the concentration difference of compounds. Therefore, theoretically, the values of RMS differences between the spectra of the same compound recorded with different spectral resolutions and/or different concentrations will be zero. [COMPARE] procedure provides three alternatives, single comparison between two spectra, comparison of spectrum of one compound with those of the registered reference compounds to find out the most probable candidate compounds of the similar spectral pattern, and finally, comparison of spectra of all registered compounds to find out the most reasonable limit of RMS value for the identity measure. In all three alternatives, the spectra of % transmittance, absorbance and derivatives up to 4th order can be compared. The results of comparisons are recorded as disk files in the form of ASCII code for the further inspection. The spectra of the most probable candidates can be shown in graphics, automatically.

Mutual Comparison of All Reference Spectra

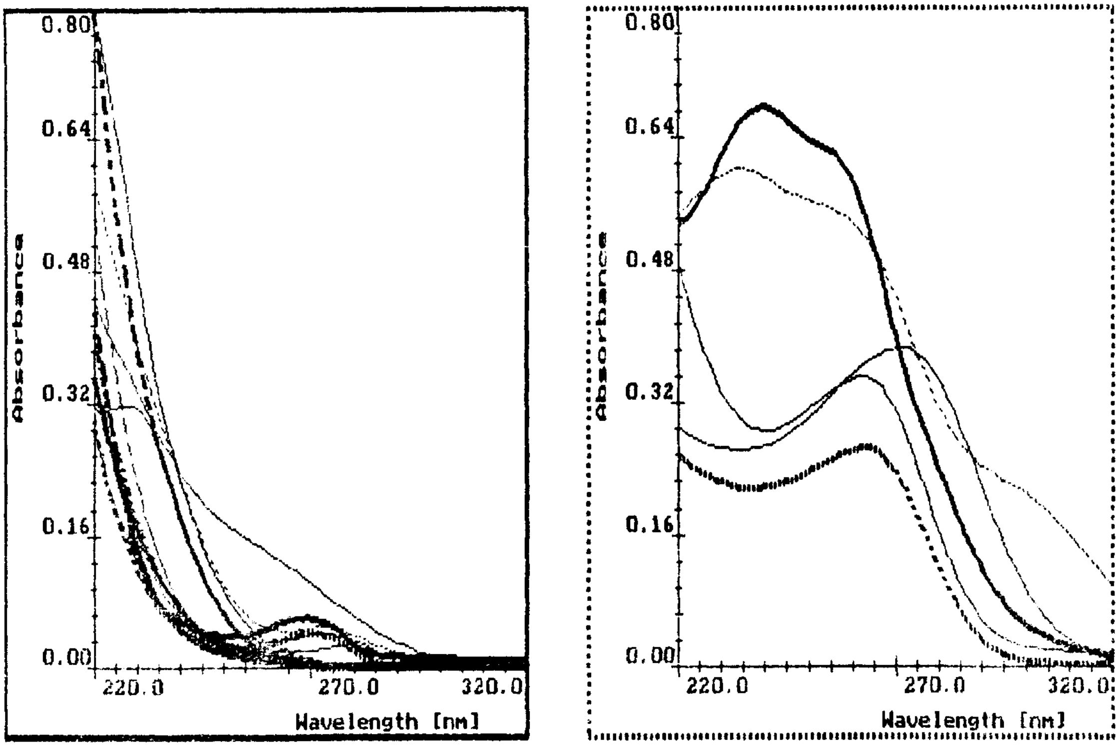
All reference spectra were compared mutually to find out the reasonable limit of RMS values for the identity measure. The number of points in the convolution block used for the data smoothing was varied to examine the influence of the degree of noise reduction on the result of comparison. Used convolution block sizes were 7, 9, 11 and 13 points. Comparison was done with absorbance and derivative spectra up to 3rd order.

Influence of Concentration Difference on Identification

The spectra of the compounds of different concentrations were compared with the reference spectra. Ampicillin trihydrate, bacampicillin, hetacillin and metampicillin were examined. These four penicillins show very similar spectra up to 2nd order derivative. Used concentrations for these compounds were of 50% and 200% of those used for reference spectra.

Influence of Contamination on Identification

Ampicillin trihydrate, bacampicillin, hetacillin and metampicillin were mixed with another peni-



(1) Penicillins

(2) Cephalosporins

Fig. 1. Absorbance Spectra of 13 Penicillines and 5 Cephalosporins.

cillins by the weight ratio of 10:1, while bacampicillin was mixed with another penicillins by the ratio of 50:1. Absorbance spectra of these mixture were acquired and compared with those of reference compounds. In addition, the derivative spectra up to 3rd order were compared.

RESULTS AND DISCUSSION

Absorbance Spectra of Examined Compounds

Absorbance spectra of 13 penicillins and 5 cephalosporins are shown in Fig. 1. As shown in the figure, most of them cannot be differentiated from each other although some of them show somewhat different patterns and/or different molar absorptivities in some spectral regions. Therefore, the complete qualitative analysis cannot be accomplished with the absorbance spectra of these compounds.

Visual Inspection of Derivative Spectra

Visual inspection of the derivative spectra was discussed in the previous report by the authors[1]. According to the visual inspection, amoxicillin trihydrate, dicloxacillin, floxacillin, penicillin V, piperacillin and 5 cephalosporins could be differentiated even by the 1st derivative spectra, showing the characteristic patterns compared to another compounds examined. Carbenicillin, carindacillin, cyclacillin and penicillin G showed characteristic pattern with 2nd derivative spectra. Ampicillin trihydrate, bacampicillin, hetacillin and metampicillin cannot be differentiated from each other even with 2nd derivative spectra. Finally, 3rd derivative spectra of these compounds showed clear differences in pattern. But the visual inspection may involve some errors and some kinds of prejudice, leading to fault conclusions. Therefore, another type of more widely acceptable method is needed as a identity measure in qualitative analysis using the derivative spectra. The RMS difference was employed as a identity measure and the applicability of it was examined.

Mutual Comparison of All Reference Spectra

Table I summarizes the result of the mutual

Table I. Result of mutual comparison of spectra of 13 penicillins and 5 cephalosporins by RMS differences

Derivative Order		0[a]	1	2	3
Number of Points in Convolution Block for Smoothing	7	18[b]	6	4	0[c]
	9	18	6	4	0
	11	18	7	4	0
	13	18	7	4	4 (0)[d]

a) Derivative Order 0: Absorbance Spectra.

b) The Number of Spectra Identified as Identical. The maximum RMS value for identity was 0.100.

c) Every spectrum can be identified as different.

d) With the maximum RMS value of 0.075, every spectrum can be identified as different.

Table II. RMS values for similar spectra of 4 penicillins

(i) Absorbance Spectra

R:C[a]	[1][b]	[2]	[3]	[4]
[1]	0.000	0.006	0.005	0.005
[2]	0.009	0.000	0.006	0.015
[3]	0.004	0.003	0.000	0.006
[4]	0.005	0.010	0.007	0.000

(ii) 1st Derivative Spectra

R:C	[1]	[2]	[3]	[4]
[1]	0.000	0.055	0.018	0.047
[2]	0.086	0.000	0.068	0.152
[3]	0.015	0.036	0.000	0.047
[4]	0.047	0.098	0.056	0.000

(iii) 2nd Derivative Spectra

R:C	[1]	[2]	[3]	[4]
[1]	0.000	0.084	0.064	0.093
[2]	0.131	0.000	0.119	0.213
[3]	0.053	0.063	0.000	0.069
[4]	0.094	0.137	0.083	0.000

(iv) 3rd Derivative Spectra

R:C	[1]	[2]	[3]	[4]
[1]	0.000	0.169	0.240	0.245
[2]	0.265	0.000	0.368	0.342
[3]	0.199	0.195	0.000	0.179
[4]	0.247	0.219	0.217	0.000

a) R: Reference Spectra; C: Compared Spectra

b) [1]: Ampicillin; [2]: Bacampicillin; [3]: Hetacillin; [4]: Metampicillin

comparison of all reference spectra of the 18 examined compounds. The maximum value of RMS differences for the identity was set to 0.100, which means that if the RMS difference between two compared spectra exceeds 0.100, these two spectra are thought to be different in pattern. With convolution block size of 7, 9 and 11 points, all compounds examined could be differentiated with 3rd derivative spectra finally, while with 13 points, 4 penicillin, which are ampicillin trihydrate, bacampicillin, hetacillin and metampicillin, could not be differentiated from each other even with 3rd derivative spectra. But with the RMS difference limit of 0.075, these 4 penicillins could be differentiated. The following data hereafter were established with the convolution block size of 9 points. Table II shows the RMS values for the similar spectra of 4 penicillins. As shown in the table, the value of RMS difference increases significantly along the order of derivatives, which demonstrates the applicability of RMS difference as a identity measure.

Influence of Concentration Difference on Identification

Table III shows the result of comparison of spectra which were acquired with the concentrations different to those used for reference spectra. All 3rd derivative spectra of the examined compounds could be identified among the reference spectra, regardless of the concentration differences.

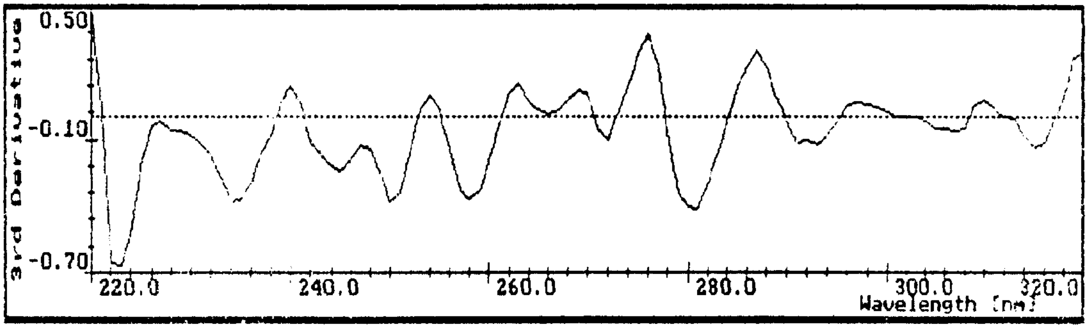
Table III. Influence of concentration difference of identification

Examined Compound	Concentration Relative to Reference (%)	Derivative Order			
		0[a]	1	2	3
Ampicillin	50	13[b]	5	3	1[c]
Bacampicillin	50	12	6	3	1
Hetacillin	50	13	5	3	1
Metampicillin	50	13	3	3	1
Ampicillin	200	11	3	1	1
Bacampicillin	200	10	1	1	1
Hetacillin	200	11	4	1	1
Metampicillin	200	11	2	1	1

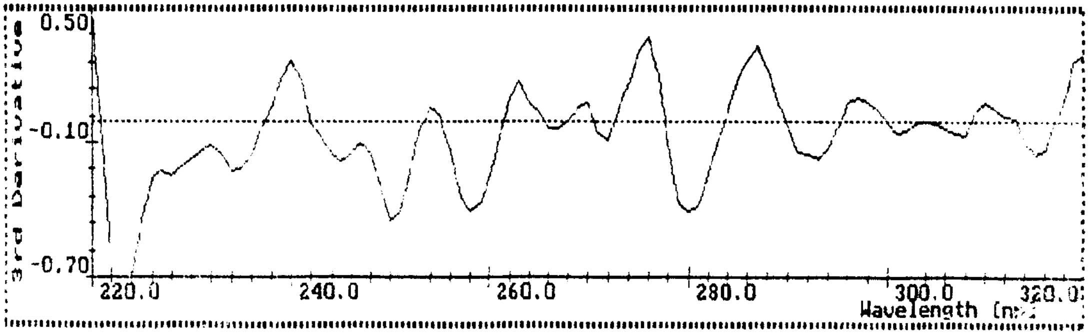
a) Derivative Order 0: Absorbance Spectra

b) The Number of Spectra Identified as Identical. The maximum RMS value for identity was 0.100.

c) Only examined compound was identified as identical, compared to reference spectra.



(1) Plot Name: Ampicillin



(2) Plot Name: Ampicillin/Amoxicillin (10:1)

Fig. 2 Third Derivative Spectra of Pure Ampicillin and the Mixture of Ampicillin and Amoxicillin (10:1).

Influence of Contamination on Identification

Fig. 2 shows the 3rd derivative spectra of pure ampicillin and the mixture of ampicillin and amoxicillin by the weight ratio of 10:1. By visual inspection, they can be perceived as identical. Table IV summarizes the result of comparisons of mixtures of this kind. As a result, only the spectra of the reference compounds identical to the majors in the mixtures were identified out of the reference spectra, regardless of the minor contaminations by other compounds.

CONCLUSION

The root mean of squares (RMS) of differences was proved to be useful as a parameter of the similarity measure for derivative spectra in the qualitative analysis of the compounds of similar structures. [SPECMAN PLUS] could be used successfully in the automated spectral analysis for the identification procedures. The concentration difference and the minor contamination did not affect the results of the identification. Automated features of the program [SPECMAN PLUS] were very useful for the qualitative analysis, presenting the users with the most probable candidates among the refer-

Table IV. Influence of contamination on identification

Major Compound	Contaminant[a]	Derivative Order			
		0[b]	1	2	3
Ampicillin	Metampicillin	13[c]	6	4	1[d]
Bacampicillin	Ampicillin	11	3	1	1
Hetacillin	Bacampicillin	13	7	4	1
Metampicillin	Hetacillin	13	4	3	1
Ampicillin	Amoxicillin	13	4	1	1
Bacampicillin	Amoxicillin	11	4	2	1
Hetacillin	Amoxicillin	13	4	3	1
Metampicillin	Amoxicillin	13	2	1	1

a) Major compound and contaminant were mixed by the ratio of 10:1.

b) Derivative Order 0: Absorbance Spectra.

c) The number of spectra identified as identical. The maximum RMS value for identify was 0. 100.

d) Only major compound can be identified as identical, compared to reference spectra.

ence spectra in auto-scaled graphical windows, which permit the users to inspect the candidate spectra. Moreover, [SPECMAN PLUS] is designed to deal with any spectral data, so the future applicability of the program is somewhat great.

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