Computer-aided spectrophotometric determination of multicomponent drugs*

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Abstract: The conditions of least squares deconvolution by linear combination have been investigated in three-component systems. The significant effects of the main factors, the lack of significant interactions and the linear relationship of absorbance with the concentration of each component were established by using a 2^3 factorial experiment design and by evaluating the results with a three-way analysis of variance. The content of active ingredients of three-component injections was determined by means of computer-aided evaluation of UV-spectra in systems that fulfilled these conditions.

Keywords: *Computer-aided UV-spectrophotometry; multicomponent drugs; least-squares deconvolution.*

Introduction

The quantitative spectrophotometric analysis of active ingredients in a solution containing more than two substances can be difficult to achieve by means of traditional methods, such as the Vierordt method. In the last two decades several mathematical methods for resolving the components in a complex spectrum have been published.

In order to resolve this problem, Blackburn applied a least-squares method based on a computer program [1]. The method allows the resolution of component spectra having overlapping peak regions; it was applied for the evaluation of a composite gamma-ray spectrum comprising the sum of different spectra, each characterising a pure radioactive element. The computer program could resolve up to 20 component spectra and could be used for any problem where the standard spectrum of each component was available.

Neuer expounded the mathematical principles of multicomponent-analysis [2], declaring that any evaluation requires some sort of fitting procedure and that the least-squares method yields the most acceptable error vector and the smallest standard deviations for the concentration values calculated. In this method the reciprocal functions become linear combinations of the single absorbances. Any fitting procedure that involves forming the reciprocal functions as a linear combination of the individual absorbances yields the same result as the least-squares method.

Horváth and Billes elaborated a method for the quantitative analysis of multicomponent systems based on the deconvolution of the UV spectrum [3]. The method of least-

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squares was applied with a wavenumber fitting routine, and weighting functions were also taken into account. It proved to be suitable for cases of extensively overlapping spectra, for extreme concentration ratios and for very small concentrations in a four-component system.

Several papers were published on the application of orthogonal polynomials for the analysis of multicomponent systems [4–6], but here only the application of the linear combination method used in this study will be discussed.

Computer-aided analysis of multicomponent systems

There are several conditions for the computer-aided analysis of multicomponent systems; some of the conditions are general, the others depend on the available software.

The conditions which must be met using the QUEST program [7] are as follows: the spectra of the pure components must be available; the relationships between the concentrations and absorbances must be linear for each component (the Lambert–Beer law must be valid); the observed spectrum of the multicomponent system must be the sum of the component spectra; the spectral data must be available in digital form.

Experimental

Solutions to be tested

The preparation to be studied was a three-component injection containing the following substances as active ingredients: drotaverine hydrochloride (32.1 mg ml^{-1}); nicotinic acid (8.8 mg ml^{-1}); phenazone (40.0 mg ml^{-1}).

The pure components were dissolved in 0.1 M hydrochloric acid; the reference solution was 0.1 M hydrochloric acid. The three-component solutions were prepared from stock solutions according to the label ratio of the components in the injection. A series of solutions was prepared according to a 2^3 factorial design, the possible errors in production being taken into account as well. The experiments were carried out within the following concentration limits: drotaverine hydrochloride (5.8–7.2 μg ml^{-1}); nicotinic acid (1.6–2.0 μg ml^{-1}); phenazone (7.3–9.0 μg ml^{-1}).

These limits were chosen assuming that the deviation from the labelled concentrations of each component in the injection did not exceed ±10% in practice.

Instrumentation

The measurements were carried out on a Perkin–Elmer Lambda 5 UV/Vis spectrophotometer interfaced with an on-line Data Station 3600 computer. For scanning, storage, retrieval and plotting the spectra, the PECUV library program [8] was used; the measurement data were evaluated with the aid of the QUEST library program [7, 9].

Measurement parameters. The principal parameters employed were: scan range (200–440 nm); data interval (1 nm); slit width (1 nm); scan speed (120 nm min^{-1}); peak threshold (0.02); abscissa scale (10 nm cm^{-1}); ordinate (min/max) (0.000–1.000); cell pathlength (1.000 cm).

Software

The Perkin–Elmer Lambda 5 UV Vis spectrophotometer interfaced to a Data Station 3600 was provided with the QUEST multicomponent-analysis program [7]. With the aid of this program package the best least-squares fitting spectrum can be obtained from the

linear combination of the components' spectra and thus the concentration of each component can be computed. The program can, in principle, resolve the spectra in a system of up to 10 components.

The form of solution is a linear combination of the standards, while the root mean square (RMS) error between the linear combination and the observed spectrum is minimised by the program. The RMS error is the sum of squares of the differences at each point of the spectrum. An unconstrained minimisation of the RMS leads to a vector equation for the solutions of the linear multipliers used to generate the best fit spectrum [9].

The matrix and the vector involved in this equation are generated by multiplying each pair of spectra together point by point and adding these values. The matrix is inverted and multiplied by the vector to generate the "solution vector", and from this, the minimum RMS error is obtained. In the example demonstrated the spectra were scanned between 200–440 nm, the data interval being 1 nm; this meant that the squares of the differences were calculated, and the evaluation of the measured spectrum was carried out at 241 nm measurement points.

Additionally the program has several options, a weighting function among them, which has the effect of diminishing the effect of concentrations that differ greatly.

Results and Discussion

### Ultraviolet spectra of the components

All three components display characteristic spectra in 0.1 M hydrochloric acid in the ultraviolet region. According to the spectrum collection of H.-W. Dibbern [10], nicotinic acid has an absorption maximum at 260 nm and phenazone at 229 nm. The absorption maxima of drotaverine hydrochloride are 354 and 241 nm (Fig. 1). Since neither nicotinic acid nor phenazone has measurable absorbance at 354 nm, drotaverine hydrochloride could be measured at this wavelength independently of the other two components. However, in the lower wavelength region, below 300 nm, the spectra of the three components overlap each other and the concentration of nicotinic acid and phenazone cannot be readily determined with confidence from the mixed spectrum by means of traditional methods.

Validity of Lambert–Beer law. Although in these experiments very dilute solutions were used, where the validity of the Lambert–Beer law can be assumed with a high degree of probability, the validity of the relationship was checked for all three substances. The regression coefficients and correlation coefficients characteristic for the linear relations are summarised in Table 1.

Conditions of the linear combination

In the absence of any interaction between the components, it may be assumed that the mixture spectrum is the linear sum of the individual spectra of the components. If the spectra of the pure components are available, the spectrum of a mixture of unknown composition can be generated as the linear combination of the individual spectra. This calculation can be carried out using the QUEST program [7] which computes the curve that best fits the measured spectrum; any other computer program calculating the best fitting curve based on linear combination is also suitable, if the spectral data are available in digital form.

Figure 1
Spectra of the components and the injection.
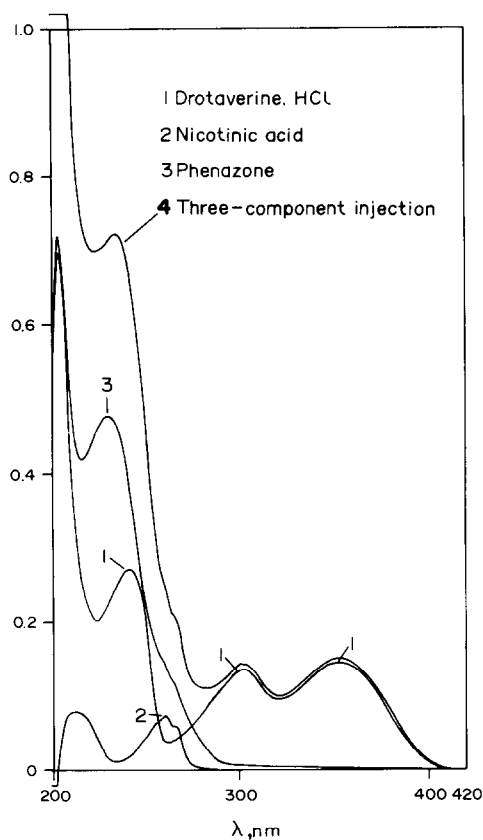
Table 1
Linear relationships between concentration and absorbance for each component

Substance	Concentration $\mu g\ ml^{-1}$	Regression coefficients $a(0)*$	$a(1)\dagger$	$r\ddagger$
Drotaverine–HCl	2–25	0.000	0.024	1.000
Nicotinic acid	2–10	0.001	0.042	0.998
Phenazone	2–16	0.000	0.059	0.999

* a(0) represents the intercept on the ordinate.
† a(1) represents the gradient.
‡ r = correlation coefficient.

Application of a 2^3 factorial experiment design. In order to establish the linear relationships operating within the presumed practical concentration limits of the injection, a 2^3 factorial experimental design was carried out. These limits were specified as concentrations differing from the nominal (label) concentration of the components in the injection by ±10%. This series of solutions contained, according to the 2^3 factorial experiment design, any possible combination of the components of a concentration differing by ±10% from the declared concentration of the injection, plus a central experiment with the concentration equal to that of the injection.

Since the pharmacopoeias usually specify a maximum deviation of the concentration of an active ingredient not exceeding ±5%, thus a difference of more than 10% is highly

improbable in practice. Thus, satisfactory measurement data in this range would provide assurance for the applicability of the method for the regular quality control of the product.

In the factorial plan of the experiments the upper level of the concentration was 10% higher, the lower level 10% lower than that of the central experiment. The 2^3 factorial plan of the experiments contains every possible combination of the components at these two concentration levels (see Table 2).

With each composition, two parallel measurements were carried out on independently prepared solutions. A set of the spectra of the pure components and of the three-component solution are illustrated in Fig. 1.

The concentrations were calculated by evaluating the spectra, using the QUEST program, as discussed above.

Evaluation of the 2^3 factorial plan. The 2^3 factorial plan of the experiments was evaluated with the aid of a three-way analysis of variance using a Hewlett-Packard library program. Table 3 shows the calculated and the tabulated values of F, convincingly proving that the measured concentration of each component is significantly influenced only by the actual concentration of the given component, and that no interaction among

Table 2
2^3 Factorial experiment design

Number of experiment	Drotaverine–HCl	Nicotinic acid	Phenazone
0	0	0	0
1	−	−	−
2	−	−	+
3	−	+	−
4	−	+	+
5	+	−	−
6	+	−	+
7	+	+	−
8	+	+	+
Central (0)	6.49 μg ml^{-1}	1.65 μg ml^{-1}	8.04 μg ml^{-1}
Lower level (−)	5.84 μg ml^{-1}	1.48 μg ml^{-1}	7.24 μg ml^{-1}
Upper level (+)	7.14 μg ml^{-1}	1.81 μg ml^{-1}	8.84 μg ml^{-1}

Table 3
Three-way analysis of variance: F-values*

Factors		Drotaverine–HCl	Measured Nicotinic acid	Phenazone
Main effects				
Concentration of drotaverine–HCl	(1)	2987	0.02	0.65
Concentration of nicotinic acid	(2)	5.30	1231	1.49
Concentration of phenazone	(3)	0.05	1.95	7546
Interactions				
12		0.45	0.32	0.55
13		1.47	0.10	0.15
23		5.97	0.54	1.94
123		4.00	0.01	0.65

*The tabulated value of F was: $F(1,8)(P = 0.95) = 5.32$.

Table 4
Evaluation of the multicomponent spectra

Number of experiment	Drotaverine–HCl Set µg ml⁻¹	measured µg ml⁻¹	Recovery %	Nicotinic acid Set µg ml⁻¹	measured µg ml⁻¹	Recovery %	Phenazone Set µg ml⁻¹	measured µg ml⁻¹	Recovery %	RMS error
0	6.49	6.32	97.4	1.65	1.63	98.8	8.04	8.20	102.0	0.002
	6.49	6.36	98.0	1.65	1.66	100.6	8.04	8.24	102.5	0.002
1	5.84	5.78	99.0	1.48	1.50	101.4	7.24	7.38	101.9	0.002
	5.84	5.77	98.8	1.48	1.50	101.4	7.24	7.32	101.1	0.002
2	5.84	5.74	98.3	1.48	1.51	102.0	8.84	8.95	101.2	0.002
	5.84	5.83	99.8	1.48	1.48	100.0	8.84	9.01	101.9	0.002
3	5.84	5.68	97.3	1.81	1.89	104.4	7.24	7.33	101.2	0.002
	5.84	5.71	97.8	1.81	1.86	102.8	7.24	7.37	101.8	0.002
4	5.84	5.68	97.3	1.81	1.86	102.8	8.84	9.02	102.0	0.002
	5.84	5.77	98.8	1.81	1.85	102.2	8.84	8.98	101.6	0.002
5	7.14	7.14	100.0	1.48	1.53	103.4	7.24	7.35	101.5	0.002
	7.14	7.05	98.7	1.48	1.49	100.7	7.24	7.35	101.5	0.002
6	7.14	6.92	96.9	1.48	1.51	102.0	8.84	8.90	100.7	0.003
	7.14	7.00	98.0	1.48	1.49	100.7	8.84	8.96	101.4	0.003
7	7.14	6.96	97.5	1.81	1.89	104.4	7.24	7.30	100.8	0.002
	7.14	6.94	97.2	1.81	1.85	102.2	7.24	7.39	102.1	0.003
8	7.14	7.03	98.5	1.81	1.86	102.8	8.84	8.99	101.7	0.002
	7.14	7.02	98.3	1.81	1.83	101.1	8.84	9.02	102.0	0.003
Mean			98.20			101.87			101.61	
Std. deviation			0.881			1.461			0.476	
Rel. std. dev. %			0.90			1.43			0.47	
Conf. interval P = 0.95			97.6–98.7			101.1–102.6			101.5–101.7	

the factors can be observed. The *F*-values characteristic for the significance of a factor are, for the significant factor, three orders higher than for the other factors, respectively for the two- and three-factor interactions, at a probability level of 0.95.

These results demonstrate the applicability of a computer program, such as QUEST, based on linear combination for evaluating the system to be tested.

Calculation of the concentrations

With the aid of the QUEST program the concentration of the components can be calculated from the stored spectral data. Although the spectra were scanned from 200 to 440 nm, the evaluation was carried out in the 220–440 nm range, since the lower wavelengths introduced a higher RMS error.

The results are summarised in Table 4, showing the set concentrations of the solutions, the calculated values and the recovery (the result of the measurement expressed as a per cent of the calculated values referred to the weighted ones). Table 4 displays the mean, standard deviation, relative standard deviation per cent and the confidence intervals of the recovery expressed in per cent.

According to the results based upon the measurement values of 2–2 parallel measurements of nine solutions containing different ratios of the components, the standard deviation of the data is within the usually accepted error for a spectrophotometric method (2%). The confidence intervals are even better: the concentration values recovered are within 97.5–102.0% of the set values for all three components, while pharmacopoeias usually accept ±5% deviation from the labelled values.

Summary

Active ingredients of a multicomponent drug can be determined by evaluating the ultraviolet spectrum scanned in the solution of their mixture, with an appropriate computer program, if the spectra of the pure components are available and the measured concentration of a given component is not significantly influenced either by the concentration of the other components present in the solution, or by interactions among the components.

A three-component injection was tested in a set of solutions prepared according to a 2^3 factorial experimental design. The evaluation of the spectra using the QUEST library program proved the measured values to be independent of the concentration ratios within ±10% of the labelled concentration of each component. The accuracy, standard deviation (less than ±1.1%) and the confidence intervals (97.5–102.0%) of the measurement data met the normal requirements of a pharmacopoeia.

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