J. Pharm. Pharmac., 1974, **26**, 682–685 *Received January* 29, 1974

Multicomponent analysis of ultraviolet spectra: application to pharmaceutical dosage forms

B. W. MADSEN AND J. S. ROBERTSON

Pharmacy Department, University of Sydney, Sydney, Australia.

Assay of sulphacetamide and physostigmine eye drops, sulphadimidine and tri-sulphonamide tablets, and a triple barbiturate capsule has been possible without prior extraction or purification of the ingredients. Concentration estimates were found using the method of least squares, and safeguards were incorporated in the computer program to determine confidence levels. The method should be applicable to other agents and dosage forms and lend itself to automation.

Previous development of a computer program for resolution of multicomponent systems in ultraviolet spectrophotometry was undertaken with known solutions of three sulphonamides, in the presence and absence of a synthetic excipient mixture (Madsen, Herbison-Evans & Robertson, 1974). However, it was felt that the final test of acceptability should be made with commercial products, and this report describes the results of tests on five different preparations.

MATERIALS AND METHODS

Commercial preparations with the following labelled characteristics were obtained: 10% sodium sulphacetamide eye drops containing sodium thiosulphate, polyvinyl alcohol, thiomersal, polysorbate 80, ethylenediaminetetraacetic acid and phosphate buffer (15 ml pack); 250 mg sulphadimidine tablets; 0·25% physostigmine salicylate eye drops with 0·25% chlorbutol preservative (15 ml pack); 500 mg triple sulphonamide tablets containing equal quantities of sulphadiazine, sulphamerazine and sulphadimidine; tri-barbiturate capsules containing 250 mg sodium barbitone, 100 mg sodium pentobarbitone and 30 mg sodium phenobarbitone.

With these assays, the intent was not to discover and quantitate any variation from batch to batch, but merely to decide whether the procedure was applicable. Dilutions were always adjusted so that absorbance readings were as large as possible, not exceeding unity. One replicate for the whole procedure (dissolution, filtration, etc.) was made for each preparation, and the spectrophotometric blank was always the dilution medium. For the barbiturate capsules, absorbance readings were made using a Varian Techtron 635 Series spectrophotometer with digital readout (Digital Multimeter, TF 2570, Marconi Instruments Ltd.). Measurements for the other four preparations were on a Perkin-Elmer 124 double beam instrument and recorder. Filtration was with a Buchner apparatus under vacuum and Whatman No. 1 paper.

Sulphadiazine, sulphamerazine, sulphadimidine and sulphacetamide were all recrystallized from ethanol: chloroform to give melting points of 255–6°, 234–8°, 197–9° and 177–9° respectively. Physostigmine salicylate and the three barbiturates were of B.P. standard. Infrared spectra confirmed identity in all cases. Reference

ultraviolet spectra for each of these active ingredients in the appropriate medium were then compiled. Provided data were drawn uniformly from the whole usable range of a spectrum, there was generally no advantage in taking more than 20–25 points. Experimental conditions for each product are shown in Table 1. The computational procedure was that of Madsen & others, (1974).

Table 1. *Experimental conditions for each of the five products.* The total weight of the solid dosage forms gives some measure of the quantity of excipient which had to be allowed for in the assay. With the first three products, all dilutions were made with the one medium, i.e. for products 1 and 2, 0·05 N NaOH and product 3, 0·05 N HCl. The last two products were first diluted in 0·05 N NaOH and subsequently in 0·05 N HCl.

Product	Total weight (approx.)	Medium (0·05N)	Dissolution, filtration and dilution	Wavelength range (nm)	Interval (nm)
1. Sulphacetamide eye drops, 10%	—	NaOH	10 ml → 1 litre of V		
		NaOH	10 ml of V → 1 litre	232–314	2
2. Sulphadimidine tablets, 250 mg	660 mg		2 tablets crushed in		
		NaOH	mortar, washings pooled & filtered → 1 litre of W		
		NaOH	25 ml of W → 1 litre	232–314	2
3. Physostigmine eye drops, 0·25%	—	HCl	10 ml → 250 ml of X		
		HCl	50 ml of X → 250 ml	232–340	4
4. Triple sulphonamide tablets, 500 mg	700 mg		3 tablets crushed in		
		NaOH	Mortar, washings pooled & filtered → 1 litre of Y		
		HCl	10 ml of Y → 1 litre	232–360	4
5. Tri-barbiturate capsules, 380 mg	400 mg		5 capsules pooled,		
		NaOH	washed & filtered → 250 ml of Z		
		HCl	25 ml of Z → 1 litre	232–284	4

RESULTS AND DISCUSSION

With the computer program described, spectral data can be analysed in two ways, linearly and non-linearly. Use of the linear treatment infers that all of the dosage form absorption can be explained by the pure spectra supplied for the regression. If there is only one active ingredient present, visual inspection of the spectrum may be sufficient to convince this is not the case. With such additional or unexpected absorption, linear estimates would be unreliable and the non-linear treatment would consequently be the one of first choice.

However, when the background is very small (but still significant) compared to the signal, or if there is more than one active ingredient present, a subjective assessment of the presence or absence of background becomes increasingly tenuous. Then it is probably best to routinely submit the data for linear analysis, when any inconsistencies will be more clearly seen for subsequent interpretation.

The results for sulphacetamide eye drops (Table 2) illustrate this point. By eye (Fig. 1), the dosage form spectra appeared compatible with that of sulphacetamide, and accordingly the linear method was used. However, the squared residuals totalled over the two solutions were not constant over the whole wavelength range, but showed a one hundred-fold increase in the region at approximately 245 nm.

Table 2. *Comparisons of stated and predicted concentrations.* The second set of figures are replicates. A represents sulphadiazine; B—sulphamerazine; C—sulphadimidine; D—sodium phenobarbitone; E—sodium pentobarbitone; F—sodium barbitone.

Product	Treatment	Label		I	II	III	Verdict
1. Sulphacetamide eye drops	linear	1·0		1·084 ± 0·3	·0050	·19	Reject
				1·088 ± 0·3	·0049	·40	
	non-linear	1·0		1·032 ± 0·9	·0014	1·94	Accept
				1.035 ± 0·9	·0014	1·67	
2. Sulphadimidine tablets	non-linear	1·25		1·35 ± 0·9	·0018	1·76	Accept
				1·32 ± 0·8	·0014	2·40	
3. Physostigmine eye drops	non-linear	2·0		1·99 ± 0·9	·0043	1·06	Accept
				2·01 ± 1·6	·0078	2·95	
4. Triple sulphonamide tablets	linear	·5	A	·520 ± 2·7	·0018	·65	Reject
				·485 ± 2·7	·0017	·87	
		·5	B	·576 ± 3·9			
				·640 ± 3·3			
		·5	C	·484 ± 2·6			
				·466 ± 2·6			
	non-linear	·5	A	·519 ± 1·7	·0011	2·05	Accept
				·491 ± 2·1	·0010	2·17	
		·5	B	·545 ± 3·3			
				·560 ± 3·1			
		·5	C	·503 ± 2·1			
				·515 ± 2·0			
5. Tri-barbiturate capsules	linear	1·5	D	1·52 ± 1·4	·0003	2·62	Accept
				1·50 ± 1·7	·0004	2·66	
		5·0	E	5·04 ± 3·1			
				5·55 ± 3·4			
		12·5	F	12·2 ± 1·1			
				11·9 ± 1·3			

I. Concn after dilution (mg per 100 ml) predicted (mean ± relative s.d. %). II ± Predicted s.d. of absorbance reading error. III Calculated Durbin-Watson d statistic.

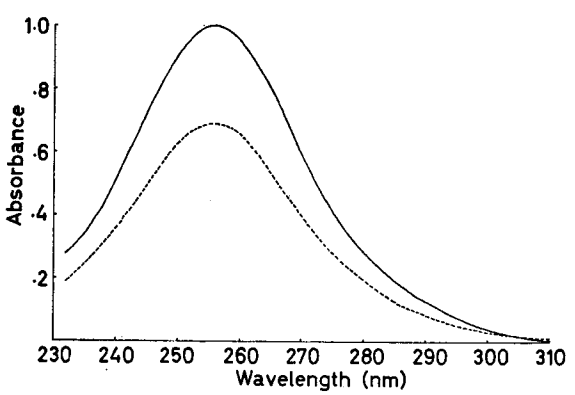

FIG. 1. Comparison of the pure and dosage form spectrum of sulphacetamide. Key ——— pure sulphacetamide,— — — — dosage form.

The very low value for the d statistic showed that one of the inherent assumptions in the method of least squares, i.e. zero correlation of residuals, was inapplicable. Also, a high value of ±0·005 for the predicted standard deviation of the absorbance reading errors indicated the fit was not good.

Therefore, the non-linear alternative was selected, initiating the program with estimates for a Gaussian approximation to this background as: mean = 256 nm and standard deviation = ± 60 nm. At the minimum of this regression, most of the deviations were thus explained with the error standard deviation significantly lowered to ± 0·0014, and the residuals randomly correlated (d = 1·94 and 1·67). For both solutions, the final background parameter estimates were approximately: peak = 0·0328 absorbance units, mean = 252 nm, standard deviation = ± 12 nm, constant term (a_0) = 0·0034.

If this two step approach does not improve the agreement between predicted and observed absorbance readings sufficiently to allow reliable concentration estimates, then there are two further possibilities worth consideration. Firstly, by reducing the wavelength range of the spectra, the probability that background absorption can be represented by a Gaussian curve increases, since typically, excipient spectra have no high frequency components and have small absorptivity (Abdine, Wahbi & Korany, 1971). Obviously, there will be a limit to how much information can be discarded to satisfy this criterion, while still producing reliable estimates. Secondly, if the main background ingredient producing the non-Gaussian shape is known (e.g. a certain preservative or sweetening agent), then inclusion of this pure spectrum may allow the program to successfully approximate the remaining excipient spectra.

Wojtowicz (1970) has previously commented on the presence of greater than 10% undeclared sulphanilamide in some sulphacetamide ophthalmic solutions. With the least squares approach illustrated in this report, a semi-qualitative test for this impurity can be made by including its spectrum, and then finding whether the concentration estimate is well defined. Even when the presence of sulphanilamide is unsuspected, the current procedure will either give an accurate estimate for sulphacetamide when the sulphanilamide content is small, or indicate rejection of the estimates since unexpected absorbance is present.

For those dosage forms with more than one active ingredient, the resulting spectrum can obviously be fitted adequately with an increasing number of combinations of the pure spectra. This means that the absorbance error standard deviation is usually small whether the regression is valid or not; acceptance or rejection decisions lean increasingly on other criteria, namely the d statistic, the individual concentration standard deviations and the agreement between replicate concentration estimates. Data for the triple sulphonamide tablets in Table 2 illustrate these features. Notice that much of the variation between successive estimates has been reduced with the non-linear treatment. For more exacting work, the means of successive concentration estimates could be used for constructing fiducial limits and probability statements on labelled contents.

The very much lower error in absorbance readings attained for the barbiturate system is probably due in large part to the digital readout. A consequence of this is that even greater resolution is possible from overlapped spectra, and that wider concentration ratios can be analysed. This means that these benefits might be expected along with the more usual ones, in any approach towards automation.

REFERENCES

ABDINE, H., WAHBI, A. M. & KORANY, M. A. (1971). *J. Pharm. Pharmac.*, **23**, 444–447.
MADSEN, B. W., HERBISON-EVANS, D. & ROBERTSON, J. S. (1974). *Ibid.*, **26**, 629–636.
WOJTOWICZ, E. J. (1970). *J. pharm. Sci.*, **59**, 240–241.