International Journal of Pharmaceutics, 51 (1989) 263–267
Elsevier

IJP 01773

Spectrophotometric assay of drugs in the presence of spectral interferants in suspensions and syrups

Chukwuenweniwe J. Eboka * and Kolawole I. Adesanya

Faculty of Pharmacy, University of Benin, Benin City (Nigeria)

(Received 11 May 1988)
(Modified version received 29 November 1988)
(Accepted 5 December 1988)

Key words: Derivative spectrophotometry; Spectral interference; Assay; Excipient; Syrup; Suspension

Summary

The zero, first and second derivative spectrophotometric techniques have been compared for the assay of drugs in the presence of spectral interferants in syrup and suspension dosage forms. Also, the standard addition method was compared with the conventional calibration method in each of the derivative spectrophotometric techniques. The results showed that the standard addition method using the second derivative technique gave acceptable results in all cases investigated.

Excipients interfere spectrally in the spectrophotometric assay of drugs in dosage forms. Therefore, extraction and clean-up of the drug (which in some cases can be cumbersome) generally precede spectrophotometric measurements of drugs in dosage forms. Derivative spectrophotometry offers a simple approach to resolving spectral overlap and interfererence (Giese and French, 1955) and can be valuable in pharmaceutical analysis (Such et al., 1980). Despite the inherent advantages of derivative spectrophotometry, only few applications to drug analyses (Such et al., 1980; Kitamura and Majina, 1983; Lawrence and Macneil, 1982; Lawrence and Kovar. 1984, 1985) and very little work on the assay of drugs in syrup and suspension dosage forms (Fell and Davidson, 1980) have been reported.

Correspondence: C.J. Eboka, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

There have been no systematic studies on the general utility of derivative spectrophotometry in the assay of drugs in the presence of excipients with which they are usually formulated. In this study, we wish to compare systematically the first and second derivative spectrophotometric techniques with the conventional zero derivative techniques in the assay of drugs in the presence of syrup and suspension excipients and dosage forms. The standard addition method (SAM) and the conventional calibration method (CM) will also be compared in each of these derivative techniques. The aim is to arrive at a method that is precise, accurate and simple for the routine assay of drugs in syrup and suspension dosage forms without resort to expensive and time wasting extraction and clean-up of the sample.

The materials used include acacia, sodium phenobarbitone, sodium lauryl sulphate and caffeine (BDH Chemicals, U.K.); tragacanth (Halewood Chemicals Ltd., U.K.,); orange oil (H.E. Daniel

TABLE 1

Percentage recoveries in the assay of drugs in the presence of syrup excipients and dosage form

Drug	Excipient brand	Standard addition method						Calibration method					
		Zero order	*n*	First order	*n*	Second order	*n*	Zero order	*n*	First order	*n*	Second order	*n*
Sodium salicylate	Syrup BP	153.50 ± 6.36	10	88.15 ± 4.82	10	100.17 ± 0.63	10	143.68 ± 5.67	10	84.57 ± 5.56	10	97.25 ± 1.60	10
Caffeine	Orange oil	128.48 ± 4.71	6	97.26 ± 0.84	6	101.12 ± 0.79	6	124.74 ± 3.37	6	96.05 ± 0.98	6	99.26 ± 0.98	6
Sodium phenobarbitone	Amaranth	113.62 ± 1.85	6	101.85 ± 0.71	6	102.00 ± 0.42	6	109.49 ± 1.18	6	101.74 ± 2.54	6	99.50 ± 1.44	6
Mean		135.80 ± 4.69	22	94.37 ± 2.50	22	100.93 ± 0.40	22	129.19 ± 4.12	22	92.39 ± 3.02	22	98.42 ± 0.87	22
Diazepam	A	127.00 ± 2.24	10	104.20 ± 2.36	10	101.01 ± 0.68	10	132.76 ± 7.84	10	100.32 ± 3.00	10	101.79 ± 1.37	10
Chloroquine phosphate	B	114.13 ± 2.36	5	99.12 ± 0.37	10	101.24 ± 0.36	10	103.46 ± 0.92	5	97.77 ± 1.12	10	99.33 ± 0.67	10
	C	115.15 ± 1.77	5	98.62 ± 0.53	10	100.33 ± 0.46	10	103.09 ± 0.65	5	96.79 ± 0.49	10	97.77 ± 0.52	10
	D	113.51 ± 2.78	5	97.76 ± 0.76	10	100.00 ± 0.68	10	111.85 ± 0.87	5	98.22 ± 1.25	10	97.08 ± 0.47	10
Paracetamol	E	115.72 ± 2.74	5	98.99 ± 0.44	5	101.66 ± 0.17	5	108.17 ± 0.93	5	102.39 ± 0.52	5	102.09 ± 0.22	5
	F	112.50 ± 1.54	5	100.17 ± 0.70	5	101.14 ± 0.50	5	107.98 ± 1.53	5	99.48 ± 1.02	5	100.68 ± 0.97	5
	G	110.99 ± 2.14	5	100.62 ± 0.39	5	101.06 ± 0.46	5	102.35 ± 0.55	5	98.09 ± 0.63	5	98.81 ± 0.73	5
Mean		117.00 ± 1.26	40	99.93 ± 0.53	55	100.82 ± 0.20	55	112.78 ± 2.70	40	98.74 ± 0.65	55	99.41 ± 0.40	55

Values are mean ± S.E.M.

Comparison of zero, first and second derivatives

Excipient:	*F*-ratio	LSD
SAM	50	8.92 [b]
CM	44	8.46 [b]
Brand	*F*-ratio	LSD
SAM	174	1.97 [c]
		1.81 [d]
CM	31	3.84 [c]
		3.52 [d]

[b] $n_1 = n_2 = 22$; [c] $n_1 = 40$, $n_2 = 55$; [d] $n_1 = n_2 = 55$

Comparison of SAM with CM

Excipient	*F*-ratio	Brand	*F*-ratio
Zero	1.1	Zero	2.2
First	0.26	First	3.6
Second	6.6	Second	3.6

Ltd., U.K.); hydrochloric acid, sulphuric acid (May & Baker, U.K.); paracetamol (Stinpex Chemicals, Nigeria); benzoylmetronidazole (Unik-Varsity Pharmaceutical Co., Nigeria); chloroquine phosphate (Afro-Arab Techni-Chemicals, Nigeria) and diazepam (Roche, Switzerland). Commercial drug preparations were purchased locally from pharmacies.

The equipment and procedure for the assays are as reported by Eboka et al. (1989) except that for liquid excipients, 10^{-3}–10^{-4}% (v/v) was used in place of 0.5 g of the solid excipient. Distilled water was used as solvent except for sodium phenobarbitone, diazepam and benzylmetronidazole where 0.1 M KOH, 0.1 M H_2SO_4 and 0.1 M HCl were used as solvents respectively. For the dosage forms, the appropriate volume containing known amount of drug was taken to prepare the sample solution.

As in tablet excipients (Eboka et al., 1989), different syrup and suspension excipients interfered spectrally in the zero derivative technique to different extents and the level of interference generally increased towards shorter wavelengths. As a result of this interference, the absorbance at the analytical wavelength for the drug was generally higher than should be, thereby giving rise to higher recoveries than the amount of drug taken both by SAM and by CM.

In the first derivative technique, the accuracies obtained when there was more than one analytical amplitude differed in certain cases depending on the level of spectral interference at these amplitudes. Generally, the results with the amplitudes at lower wavelengths were less accurate due to the higher degree of interference. For example, the first derivative assay, using SAM and CM respectively, gave for sodium salicylate in syrup BP 72.61 ± 3.61 and 68.90 ± 3.94; for diazepam in syrup dosage form 109.50 ± 2.85 and 96.38 ± 5.70 and for benzoylmetronidazole in suspension dosage form 86.40 ± 1.57 and 82.98 ± 1.38 using the peak at 228 nm in each case. However, the corresponding results at higher wavelengths are 99.68 ± 0.86 and 100.24 ± 0.74 (at 294 nm) for sodium salicylate; 98.91 ± 1.68 and 100.27 ± 0.41 (at 274 nm) for diazepam; and 94.06 ± 0.24 and 88.78 ± 1.18 (at 272 nm) for benzoylmetronidazole respectively. Such inaccuracies in the first derivative arose when it was not possible to reduce the interference substantially. The second derivative technique reduced these interferences substantially and high inaccuracies were not observed with it.

Analysis of variance was used to compare the results obtained by the different derivative techniques and also used to compare SAM with CM. If a significant difference was established in the comparison of the derivative techniques the least significant difference (LSD) procedure for multiple comparison was then used to determine which ones were different. Results of the assays of drugs in the presence of syrup excipients and dosage form are shown in Table 1. Analysis of variance of the raw data for the excipients showed that differences between the 3 derivative techniques were significant both by SAM and CM as their respective F-ratios were larger than $F_{2,63,0.05} = 3.15$. The least significant difference (LSD) procedure for multiple comparisons showed that by SAM, the first derivative mean (94.37 ± 2.50 (S.E.M.)) and the second derivative mean (100.93 ± 0.40) were individually different from the zero derivative mean (135.80 ± 4.69), but the first and the second derivative means were not significantly different from each other. Similarly in CM, the first derivative mean (92.39 ± 3.08) was not different from the second derivative mean (98.42 ± 0.87) but both were different from the zero derivative mean (129.19 ± 4.12). Thus in both methods, the first and second derivatives were not different from one another but each was significantly different from the zero derivative. Comparison of SAM and CM showed that in the zero and first derivatives, there were no significant differences but in the second derivative, SAM (100.93 ± 0.40) was better than CM (98.42 ± 0.87) as the F-ratio is greater than $F_{1,42,0.05} = 4.08$.

The trends observed in assays in syrup dosage form were similar to those observed with syrup excipients, i.e., both first and second derivative techniques were individually better than the zero derivative technique (SAM mean values: 99.93 ± 0.53, 100.82 ± 0.20 and 117.00 ± 1.26 respectively, and CM mean values: 98.74 ± 0.65, 99.41 ± 0.40 and 112.78 ± 2.70, respectively); but there was no difference between the first and the second deriva-

TABLE 2

Percentage recoveries in the assay of drugs in the presence of suspension excipients and dosage form

Drugs	Excipient brand	Standard addition method						Calibration					
		Zero order	*n*	First order	*n*	Second order	*n*	Zero order	*n*	First order	*n*	Second order	*n*
Sodium phenobarbitone	Acacia	131.17 ± 12.23	6	97.98 ± 1.23	6	101.42 ± 0.49	6	127.87 ± 9.45	6	98.25 ± 1.91	6	101.32 ± 1.25	6
Chloroquine phosphate	Acacia	108.10 ± 1.07	6	97.92 ± 0.71	12	101.77 ± 0.79	12	105.98 ± 1.41	6	88.69 ± 1.47	12	92.55 ± 0.94	12
Sodium salicylate	Sodium Lauryl Sulphate	127.85 ± 4.02	12	98.54 ± 0.96	12	99.99 ± 0.58	12	125.63 ± 4.54	10	100.38 ± 1.10	12	99.09 ± 1.12	12
	Syrup BP	153.50 ± 6.36	10	88.15 ± 4.82	10	100.17 ± 0.63	10	143.68 ± 5.67	12	84.57 ± 5.56	10	97.25 ± 1.60	12
Caffeine	Orange oil	128.48 ± 4.71	6	97.26 ± 0.84	6	101.12 ± 0.80	6	124.75 ± 3.38	6	96.05 ± 0.98	6	99.24 ± 0.97	6
	Tween 80	188.45 ± 20.00	6	88.55 ± 0.68	6	101.79 ± 0.41	6	188.57 ± 18.57	6	89.01 ± 1.45	6	100.46 ± 0.50	6
Sodium phenobarbitone	Amaranth	113.62 ± 1.85	6	101.85 ± 0.71	6	102.00 ± 0.42	6	109.49 ± 1.18	6	101.74 ± 2.54	6	99.50 ± 1.44	6
Chloroquine phosphate	Tragacanth	109.99 ± 1.31	6	97.79 ± 0.72	12	100.56 ± 0.49	12	105.70 ± 0.55	6	96.72 ± 0.52	12	96.46 ± 0.38	12
Mean		133.59 ± 4.09	58	96.38 ± 0.90	70	100.96 ± 0.24	70	129.32 ± 3.87	58	94.11 ± 1.13	70	97.62 ± 0.50	70
Benzoylmetronidazole	A	104.01 ± 0.40	10	90.23 ± 1.48	10	97.75 ± 0.24	5	101.75 ± 0.53	10	85.63 ± 1.23	10	92.69 ± 0.87	5
Paracetamol	B	107.77 ± 0.97	6	94.99 ± 0.81	6	96.47 ± 0.57	6	95.23 ± 0.65	6	91.94 ± 0.67	6	90.02 ± 0.69	6
Mean		105.42 ± 0.63	16	92.02 ± 1.12	16	97.06 ± 0.37	11	99.30 ± 0.91	16	87.85 ± 1.08	16	91.23 ± 0.67	11

Comparison of zero, first and second derivatives

Excipient	*F*-ratio	LSD	Brand	*F*-ratio	LSD
SAM	126	4.94 [e]	SAM	71	2.29 [g]
		4.71			2.54
CM	80	5.94 [e]	CM	42	2.60 [g]
		5.65 [f]			2.88 [h]

[e] $n_1 = 58$, $n_2 = 70$; [f] $n_1 = n_2 = 70$; [g] $n_1 = n_2 = 16$; [h] $n_1 = 16$, $n_2 = 11$

Comparison of SAM with CM

Excipient	*F*-ratio	Brand	*F*-ratio
Zero	0.57	Zero	31
First	2.5	First	7.2
Second	37	Second	58

tives in both SAM and CM. Also, there were no differences between SAM and CM in the first and zero derivatives but in the second derivative, there was a significant difference.

The results for the assays in the presence of suspension excipients and dosage form are shown in Table 2. For the excipients, the trend was the same as for syrup excipients and dosage form: first and second derivative techniques were not different from one another but each was individually better than the zero derivative in SAM (96.38 $\pm$ 0.90, 100.96 $\pm$ 0.24 and 133.59 $\pm$ 4.09, respectively) and in CM (94.11 $\pm$ 1.13, 97.62 $\pm$ 0.50 and 129.32 $\pm$ 3.87, respectively). Also in comparing SAM with CM, there were no differences in the zero and first derivatives but in the second derivative, SAM (100.96 $\pm$ 0.24) was better than CM (97.62 $\pm$ 0.50). For the suspension dosage form, all 3 derivative techniques were significantly different from each other both in SAM (105.42 $\pm$ 0.63, 92.02 $\pm$ 1.12 and 97.06 $\pm$ 0.37) and in CM (99.30 $\pm$ 0.91, 87.85 $\pm$ 1.08 and 91.23 $\pm$ 0.67) for the zero, first and second derivative techniques respectively. Note that the trend for the recoveries followed the same order: zero > second > first both in SAM and CM. In comparing SAM and CM, there was significant difference in all 3 derivative techniques. In the zero derivative, CM (99.30 $\pm$ 0.91) was better than SAM (105.42 $\pm$ 0.63); in the first derivative, SAM (92.02 $\pm$ 1.12) was better than CM (87.85 $\pm$ 1.08) while in the second derivative SAM (97.06 $\pm$ 0.37) was better than CM (91.23 $\pm$ 0.67). The poor result obtained for suspension dosage form in comparison with those obtained in other dosage forms and in the presence of excipients was caused by the high quantity of excipients present, the high viscosity and turbidity of the preparations which made it difficult to measure exact volumes of the suspensions.

It was further observed that generally the results obtained with the second derivative in the presence of the excipients and dosage forms (including tablets) and in both SAM and CM, were more precise than those of the first and zero derivatives. This generally led to smaller within-set differences and hence statistically significant differences were observed in all the cases investigated using the second derivative when CM was compared with SAM. In all these cases, the accuracy of SAM was superior to CM using the second derivative technique. The poor precision in the first and zero derivative techniques may actually be responsible for the differences between their SAM and CM that are not statistically significant in some cases.

In conclusion, the second derivative technique generally gave more accurate and precise results in both SAM and CM than the first and zero derivative techniques in the assay of drugs in the presence of excipients and in dosage forms. However, the accuracy of SAM in the second derivative has been shown to be superior to the corresponding CM, and as it gave excellent results in all the cases investigated, it can therefore be used for the assay of drugs in the presence of excipients and in dosage forms without resort to extensive extraction and clean-up. The values obtained with SAM in the second derivative for all the drugs investigated here, fell within the generally acceptable limits for syrups and suspensions (United States Pharmacopeia, 1980).

References

Eboka, C.J. and Adesanya, K.I., Assay of drugs in the presence of spectral interferants in tablets. *Int. J. Pharm.*, 51 (1989) 161–168.

Fell, A.F. and Davidson, A.G., The determination of sulphoxide in degraded chlorpromazine formulation by second derivative UV-spectrophotometry. *J. Pharm. Pharmacol.*, 32 Suppl. (1980) 97p.

Giese, A.T. and French, C.S., The analysis of overlapping spectral absorption band by derivative spectrophotometry. *Appl. Spectrosc.*, 9 (1955) 78–94.

Kitamura, K. and Majina, R., Determination of salicylic acid in aspirin powder by second derivative ultraviolet spectrometry. *Anal. Chem.*, 55 (1983) 54–56.

Lawrence, A.H. and Macneil, J.D., Identification of amphetamine and related illicit drugs by second derivative ultraviolet spectrometry. *Anal. Chem.*, 54 (1982) 2385–2387.

Lawrence, A.H. and Kovar, J., Analysis of heroin-morphine mixtures by zero order and second derivative ultraviolet spectrometry. *Anal. Chem.*, 56 (1984) 1731–1734.

Lawrence, A.H. and Kovar, J., Analysis of Δ^9-tetrahydrocannabinol–cannabinol mixtures by second derivative ultraviolet spectrometry. *Analyst*, 110 (1985) 827–830.

Such, V., Traveset, J., Gonzalo, R. and Gelpi, E., Stability assays of aged pharmaceutical formulas for thiamine and pyridoxine by high performance thin-layer chromatography and derivative spectrometry. *Anal. Chem.*, 52 (1980) 412–419.

United States Pharmacopeia, Twentieth Revision, United States Pharmacopeial Convention, Inc. Rockville Md., 1980, pp. 12, 229, 609, 615, 621, 749 and 787.