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Quantitative estimation of salbutamol sulphate by derivative UV spectroscopy in the presence of albumin

G. Mukherji [1] and N. Aggarwal [2]

Department of Pharmacy, Jamia Hamdard, New Delhi (India)

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Summary

Salbutamol sulphate (SS) can be analysed very accurately by second-derivative (^{2}D) UV spectroscopy at concentrations between 1 and 80 μg/ml, in the presence of fairly high levels of bovine albumin. The non-derivatised (^{0}D) UV-spectroscopic analysis of salbutamol sulphate is severely hindered due to interference by albumin while in ^{4}D, Beer-Lambert's law is not obeyed between 1 and 8 μg/ml.

Introduction

Derivative spectroscopy can effectively resolve many analytical problems, including resolution of multi-component systems, removal of sample turbidity and matrix background, and enhancement of spectral details. This technique can be successfully applied in qualitative and quantitative analysis in pharmaceutical (Traveset et al., 1980; Davidson and Elshiekh, 1982), clinical (O'Haver, 1979) and biomedical (Matsushima et al., 1975) areas.

The derivative UV spectroscopic technique in second (^{2}D) and fourth (^{4}D) orders has been effectively used in the analysis of ephedrine and pseudoephedrine in pharmaceutical formulations, including elixirs, mixtures, nasal drops and tablets (Davidson and Elshiekh, 1982). Simultaneous determinations of naphazoline and diphenhydramine HCl in nasal drops (Santoni et al., 1989), ephedrine HCl and diphenhydramine HCl (Korany et al., 1986), and salicylic acid in aspirin powder (Kitamura and Majima, 1983), can be performed by ^{2}D spectrometry. Analysis of diazepam and oxazepam in dosage forms by ^{4}D spectrophotometry (Abdel-Hamid and Abuirjeie, 1988) and estimation of drugs by other orders of derivatives have also been reported (Tobias, 1983; Fasanmade and Fell, 1985; Korany et al., 1986).

In the field of biological and biochemical estimations, Ichikawa (1977) used ^{2}D spectrophotometry as an effective tool for examining pheny-

Correspondence to: G. Mukherji, E-12/31, DLF-Qutab Enclave (Phase-1), Gurgaon 122 001, India.
[1] *Present address:* College of Pharmacy, SGSITS, Indore, M.P., India.
[2] *Present address:* E4, I.T.I., Pusa Campus, New Delhi 12, India.

lalanine residues in proteins, and the determination of bilirubin in the presence of albumin (Cook et al., 1977) by ^{1}D spectroscopy has been reported. Techniques for the elimination of broad background absorbance in the determination of small amounts of amphetamine in liver extract by ^{2}D spectroscopy (Gill et al., 1982) and of nitrofurantoin in urine by derivative spectroscopy (Poulou and Macheras, 1986) suggest an alternative procedure for drug analysis in in vivo samples.

Substances like gelatin and albumin are often used as excipients in drug delivery systems, especially the novel DDS. In the quantitative estimation of a drug present in such a formulation, the excipients often interfere by being present in the analysing solution in minute quantities. This necessitates the use of isolation techniques such as chromatography for the drug prior to its analysis. The derivative form of spectrophotometry being suitable for the analysis of multi-component mixtures, the estimation of salbutamol sulphate in the presence of albumin has been carried out by second and fourth order analysis and compared with that of the zero-order determination. This technique can be utilized, either directly or after suitable modifications, in the estimation of salbutamol sulphate in in vivo samples having a lower proportion of albumin, whose limiting concentration in relation to the drug has been determined by experimentation here.

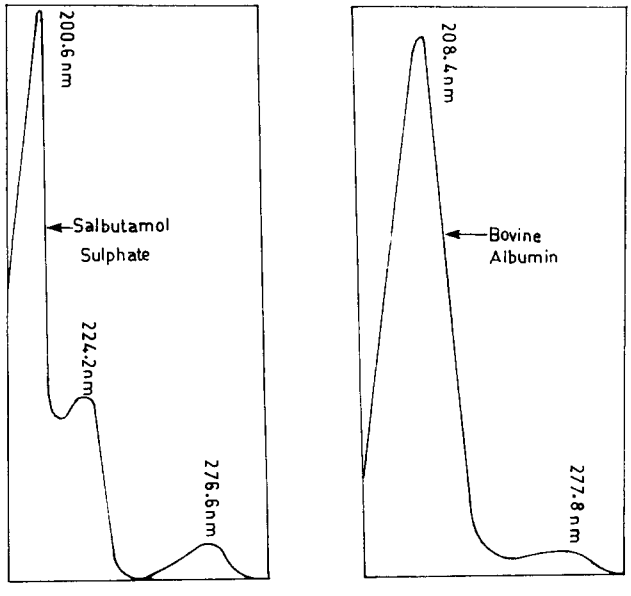

Fig. 1. UV spectra of salbutamol sulphate and bovine albumin.

Experimental

Materials and equipment

Salbutamol sulphate (Ranbaxy Labs, New Delhi) and bovine albumin (Sigma Chemical Co.) were used. A Shimadzu UV-260 double-beam spectrophotometer was employed for the analysis.

Method

The following aqueous solutions of different compositions were prepared:

(a) salbutamol sulphate at 1, 2,..., 8 μg/ml, and 10, 20,..., 80 μg/ml;
(b) salbutamol sulphate at 1, 2,..., 8 μg/ml and a fixed albumin concentration of 1 μg/ml;
(c) a fixed salbutamol sulphate concentration of 2 μg/ml and varying albumin concentrations of 1, 2,..., 8 μg/ml;
(d) a fixed salbutamol sulphate concentration of 20 μg/ml and varying albumin concentrations of 1, 2,..., 8 μg/ml and 10, 20,..., 80 μg/ml.

Zero-order (^{0}D), second-order (^{2}D) and fourth-order (^{4}D) UV absorption spectra were recorded for each solution with water being used as the reference solution. The absorbance was noted at 224.2 nm for ^{0}D. The amplitude of the curve lying between 236 nm (peak) and 227 nm (valley) for ^{2}D, and that between 236 nm (valley) and 228 nm (peak) for ^{4}D were measured. Results of six determinations are shown. The order of the derivative (0, 2, 4) is indicated wherever appropriate by the corresponding numerical superscript. The correlation coefficient, r, regression equation and t value are designated by the subscripts SS or A. For example, for ^{0}D UV analysis of solutions containing varying concentrations of albumin and a fixed level of salbutamol sulphate, the correlation coefficient is denoted as $^{0}r_{A}$. An error of less than 1.0% was

considered acceptable for analysis. The regression equation is expressed as:

$$y = \bar{y} + b(x - \bar{x}),$$

where $\bar{x}$ is the mean of the concentration of salbutamol sulphate or albumin, $\bar{y}$ denotes the mean of the absorbance or amplitude corresponding to x and b is the regression coefficient. Evaluation of the correlation coefficient (H_0: $\rho = 1$) was performed by t-test:

$$t = (r - 1) \div \left[(1 - r^2)(N - 2)\right]^{1/2},$$

where N is the number of samples. Significance was determined at $P = 0.05$.

Results and Discussion

A full-range UV spectrum (^{0}D) of salbutamol sulphate shows three absorption maxima at 200.6, 224.2 and 276.6 nm while that of albumin exhibits two – at 208.4 and 277.8 nm (Fig. 1). The peak at 224.2 nm for the drug is therefore very suitable for analysis in the presence of albumin. Since the maximum at 208.4 nm for albumin is broad at the base and extends between 180–240 nm, the quantitative estimation of salbutamol sulphate at 224.2 nm by simple, non-derivative (^{0}D) UV spectroscopy entails errors due to interference by the former.

Fig. 2 shows the concentration-related profiles of salbutamol sulphate between 10 and 80 μg/ml

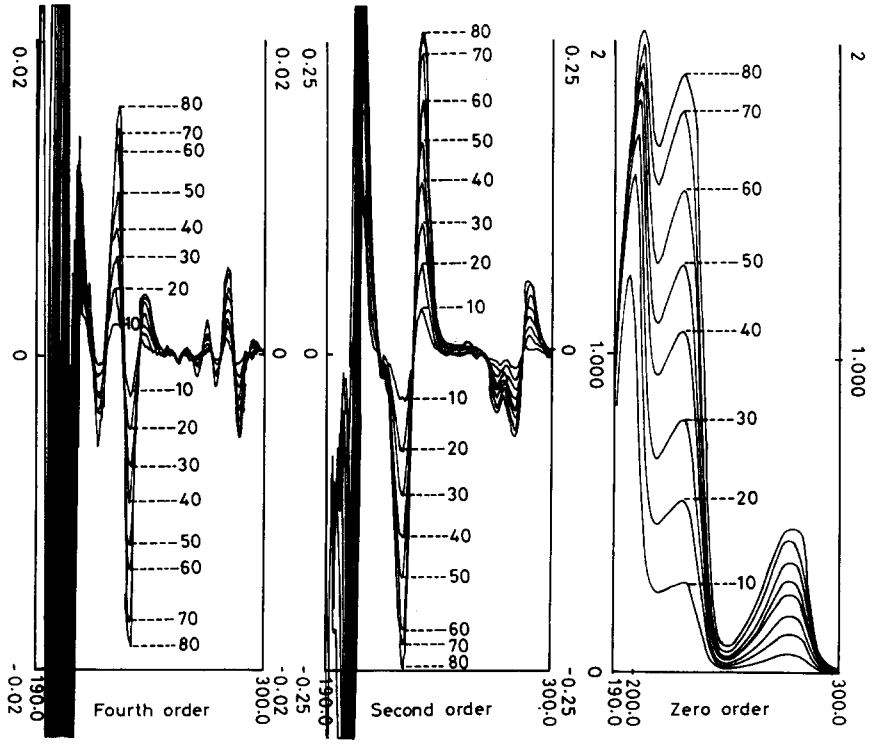

Fig. 2. Spectra showing absorbance/amplitude of salbutamol sulphate (10–80 μg/ml) for plotting of ^{0}D, ^{2}D and ^{4}D standard curves of the drug.

TABLE 1

Mean of observations of absorbances (^0D) / amplitudes (^2D) of solutions containing a fixed amount of albumin (1.0 μg/ml) and varying concentrations of salbutamol sulphate (1.0–8.0 μg/ml)

Concentration of salbutamol sulphate (μg/ml)	^0D		^2D	
	Absorbance	% error	Amplitude (cm)	% error
1.0	0.0448	31.47	2.54	1.09
2.0	0.0894	6.43	4.23	0.92
4.0	0.1299	3.62	7.80	0.26
6.0	0.1866	3.43	11.87	0.92
8.0	0.2466	0.45	15.60	0.45
Mean % error		9.08		0.73

as determined by ^0D, ^2D and ^4D UV spectroscopy. On transformation of the data into standard curves, it was observed that Beer-Lambert's law was followed in each of the three cases. However, for drug solutions of 1–8 μg/ml, ^4D fails to relate concentration with amplitude. The ^4D spectra show fine splitting of the peaks between 228 and 236 nm, presumably due to low signal and high noise effects and hence the amplitudes cannot be measured. The standard curves of the drug at ^0D and ^2D show linearity, as depicted in Fig. 3.

Following the preparation of standard curves for salbutamol sulphate, the drug was analysed between 1 and 8 μg/ml, in the presence of albumin at a fixed concentration of 1 μg/ml. The results are listed in Table 1. For an error of less than 1%, 2 μg/ml of salbutamol sulphate can be determined by ^2D UV for a drug-albumin ratio of 2:1. The correlation coefficient, $^2r_{SS}$, was found to be 0.9996, and the best-fit line as equation can be expressed as $^2y_{SS} = 8.4086 + 1.8794(x - 4.2)$. At $N = 5$, $^2t_{SS} = -0.025$, which is not a significant correlation. By ^0D UV, the minimum analyzable concentration of salbutamol sulphate is 8 μg/ml (drug : albumin = 8 : 1) [$^0r_{SS} = 0.9964$; $^0y_{SS} = 0.1395 + 0.0277(x - 4.2)$; $^0t_{SS} = -0.074$ at $N = 5$ – not significant]. A low signal-to-noise ratio accounted for the failure of analysis by ^4D UV.

In another study, solutions having a fixed concentration of salbutamol sulphate (2 μg/ml) and varying concentrations of albumin (1–8 μg/ml) were analysed by ^0D and ^2D UV. The data obtained demonstrate an increase in percentage er-

TABLE 2

Absorbances (^0D) / amplitudes (^2D) of solutions containing a fixed amount of salbutamol sulphate (2 μg/ml) and varying concentrations of albumin (1–8 μg/ml)

Concentration of albumin (μg/ml)	Zero order		Second order	
	Absorbance ± S.D.	% error	Amplitude (cm) ± S.D.	% error
1	0.0730 ± 0.0064	11.11	4.3000 ± 0.1314	0.65
2	0.0788 ± 0.0052	19.90	4.4250 ± 0.1990	3.58
3	0.0844 ± 0.0022	28.42	4.7100 ± 0.2040	10.25
4	0.0864 ± 0.0021	31.47	4.9750 ± 0.1758	16.45
5	0.0911 ± 0.0040	38.62	5.0200 ± 0.0721	17.50
6	0.0929 ± 0.0033	41.36	5.2300 ± 0.0505	22.42
7	0.1037 ± 0.0040	57.74	5.3300 ± 0.1603	24.76
8	0.1088 ± 0.0020	65.63	5.4800 ± 0.7460	28.27
Mean % error		36.78		15.48

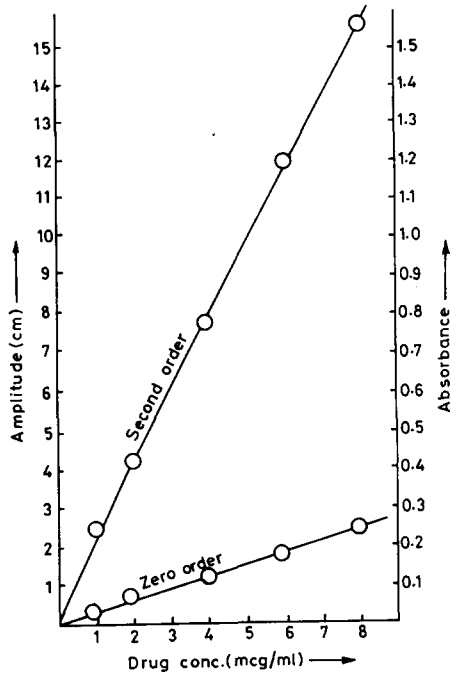

Fig. 3. Standard curves of salbutamol sulphate in zero and second orders.

ror with increase in albumin concentration (Table 2). The afore-mentioned finding was confirmed here, in that only 1 μg/ml of albumin can be present in solution with 2 μg/ml of the drug if the level of interference is to be restricted within 1% in ^{2}D. Correlation coefficient values for both ^{0}D and ^{2}D UV showed a positive correlation between absorbance/amplitude and concentration of albumin in solution [$^0r_A = 0.9842$; $^0y_A = 0.0899 + 0.0048(x - 4.5)$; $^0t_A = -0.2186$ at $N = 8$ – not a significant; $^2r_A = 0.9879$; $^2y_A = 4.9337 + 0.1713(x - 4.5)$; $^2t_A = -0.1911$ at $N = 8$ – not significant].

Spectra of solutions containing a fixed quantity of salbutamol sulphate and varying amounts of albumin should merge with each other to overlap perfectly if there is no interference from the latter. Analysis of solutions containing salbutamol sulphate (20 μg/ml) and albumin (1–8 μg/ml) showed the merging of bands in ^{2}D and ^{4}D. Thus, ^{2}D and ^{4}D UV estimations permit the analysis of salbutamol sulphate in the presence of up to 8 μg/ml of albumin. The absorbance values for ^{0}D, showing minimum and maximum percentage errors of 0.9 and 13.99%, permit a maximum level of only 1 μg/ml of albumin in solution for the analysis of 20 μg/ml of salbutamol sulphate [$^0r_A = 0.9883$; $^0y_A = 0.5632 + 0.0101(x - 4.5)$; $^0t_A = -0.188$ at $N = 8$ – not significant].

Spectra of solutions containing 20 μg/ml of salbutamol sulphate and increasing levels of albumin (10–80 μg/ml) demonstrated that the percentage error was directly proportional to the albumin concentration (Table 3). A maximum of 20.0 μg/ml of albumin can be permitted in solutions containing 20 μg/ml of salbutamol sulphate by ^{2}D and ^{4}D [$^0r_A = 0.9915$; $^0y_A = 0.8358 + 0.0066(x - 45)$; $^0t_A = -0.16$ at $N = 8$ – not signif-

TABLE 3

Absorbances (^{0}D)/amplitudes (^{2}D, ^{4}D) of solutions containing a constant amount of salbutamol sulphate (20 μg/ml) and varying concentrations of albumin (10–80 μg/ml)

Concentration of albumin (μg/ml)	Zero order		Second order		Fourth order	
	Absorbance ± S.D.	% error	Amplitude ± S.D. (cm)	% error	Amplitude ± S.D. (cm)	% error
10	0.6088 ± 0.0356	15.18	4.4600 ± 0.0338	0.25	3.4500 ± 0.0479	0.12
20	0.6741 ± 0.0385	27.53	4.4900 ± 0.0779	0.92	3.4610 ± 0.0820	0.44
30	0.7044 ± 0.0551	33.27	4.5100 ± 0.0877	1.37	3.5270 ± 0.2609	2.34
40	0.8158 ± 0.0524	54.33	4.5980 ± 0.1440	3.35	3.7460 ± 0.2274	8.70
50	0.8857 ± 0.0498	67.57	4.6900 ± 0.1606	5.47	3.7880 ± 0.0942	9.92
60	0.9494 ± 0.0594	79.62	4.8850 ± 0.2670	9.80	3.9250 ± 0.1118	13.90
70	1.0005 ± 0.0401	89.28	5.0856 ± 0.3092	14.33	4.0160 ± 0.3060	16.54
80	1.0474 ± 0.0412	98.16	5.2675 ± 0.2050	18.40	4.1000 ± 0.2449	18.98
Mean % error		58.12		6.73		8.87

icant; ${}^2r_A = 0.9521$; ${}^2y_A = 4.7485 + 0.1173(x - 45)$; ${}^2t_A = -0.384$ – not significant; ${}^4r_A = 0.9861$; ${}^4y_A = 3.7516 + 0.0102(x - 45)$; ${}^4t_A = -0.205$ at $N = 8$ – not significant].

Conclusion

Based on the derivative-UV estimation of salbutamol sulphate in the presence of albumin, it can be concluded that, while ^{0}D UV showed positive interference by albumin, there was a marked improvement with ^{2}D. By ^{2}D UV, the sensitivity achieved for the analysis is as low as 2 μg/ml, in the presence of as much as 1 μg/ml of albumin. For a higher level of 20 μg/ml of the drug, interference from 20 μg/ml of albumin does not raise the error beyond the set level of 1%. ^{4}D UV cannot be employed for the analysis of salbutamol sulphate at concentrations lower than 8 μg/ml, whereas, for higher levels of the drug, the observations are similar to those of ^{2}D UV.

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