UV SPECTROPHOTOMETRIC DETERMINATION OF SUBMICRO QUANTITIES OF SERPENTINE IN SERPAJMALINE

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The absorbance of serpentine in 5N acetic acid at 307 mμ has been used for the submicro spectrophotometric determination of this substance in 'Serpajmaline' which is predominantly a mixture of serpentine, serpentinine and ajmaline. Serpentinine which also absorbs at 307 mμ is separated from the complex through electrophoresis. The extent of interference due to ajmaline in the UV absorbance measurements of serpentine has been determined. The method is accurate within ±1-2%.

Introduction

Serpajmaline[1] is a Rauwolfia alkaloidal fraction consisting mainly of serpentine, serpentinine and ajmaline alkaloids. It has been used against cardiac disorders and is, therefore, clinically important. In view of its therapeutic activity, it is essential that the exact amounts of its constituent alkaloids should be known reliably.

Accordingly, this communication describes a spectrophotometric method for the determination of the amount of serpentine in serpajmaline. In literature, Machovicova *et al.*[2] have separated a mixture of ajmaline, serpentine and reserpine by paper chromatography and have determined colorimetrically the individual alkaloids by their formation of addition compounds with methyl orange. Sahli [3] has estimated Rauwolfia alkaloids after their chromatographic and electrophoretic separations. However, the interference due to serpentinine and ajmaline, when present with serpentine, has not been investigated. Serpentine and serpentinine are present in serpajmaline in comparatively large amounts and although separation of serpentine from ajmaline can be effected by paper chromatography,[3,4] serpentine and serpentinine spots are superimposed and it is difficult to estimate serpentine in the presence of comparatively larger amount of serpentinine by UV measurements.

In our method serpentinine is separated from serpajmaline by paper electrophoresis which resolves the fraction into two components viz. serpentinine and serpentine and ajmaline. Serpentine and ajmaline thus freed from serpentinine are determined after elution from the electrophoretic paper by measuring UV absorbance at 307 mμ in 5N acetic acid ($E^{1\%}_{1\ cm.}$ of serpentine $=560$. $E^{1\%}_{1\ cm.}$ of ajmaline $=35$.)

Since the contamination due to ajmaline which is a weak UV absorbant, cannot be avoided as the electrophoretic R_f values of ajmaline and serpentine are the same, the extent to which ajmaline interferes with the estimation of serpentine has, therefore, been measured and accounted for in the determination.

Materials and Methods

Materials.—(1) Unicam SP500 spectrophotometer with 1 cm cells. (2) Serpentine stock solution 0.1% in 5N acetic acid. (3) Ajmaline solution 0.1% in 5N acetic acid. (4) Serpajmaline solution 0.4% in alcohol. (5) Electrophoresis apparatus (Durrum) FB11.

The electrophoresis chamber is constructed according to 'Durrum', where filter paper strips are suspended tent-like over a rack. The ends of the strips are immersed in 5N acetic acid contained in two buffer vessels in which two long platinum wire electrodes are embedded. The two buffer vessels are built in the compact acid proof lower part to which the paper holder is firmly screwed. A tight fitting transparant plastic cover makes the chamber air-tight. The filter paper strips used throughout this work, are Whatman No. 1 chromatographic paper. The paper is pre-washed for 48 hours in 5N acetic acid by downward capillary flow in a chromatographic tank.

Preparation of Calibration Curves.—The stock solution of serpentine (20, 40, 80 and 100 μl) was diluted to 10 ml. with 5N acetic acid. The absorbance of these solutions was measured in a 1 cm. cell, using 5N acetic acid as blank at 307 mμ. The calibration curve obtained was a straight line.

Assay of Serpajmaline Complex.—The solution of serpajmaline in ethanol was applied to the prewashed paper strip as a spot containing 80-100μg. of the complex with an 'Agla' micrometer syringe. The solvent was evaporated with the help of a

hair drier during the application of the solution to prevent the spot from spreading. The strip was placed in position in the apparatus and the solvent was allowed to ascend to the apex of the strip by capillary action. A blank strip was placed along with the spotted strip. The cover was replaced and 380 volts was applied across the strips for 4 hours. The strips were removed from the cabinet and dried in air. The serpentine zone having blue flourescence was marked with a lead pencil and the zone on the sample strip was cut along the marked line. A piece was cut from the blank strip corresponding in size and position to the one carrying serpentine. The blank and the sample pieces were reduced into bits and were placed overnight in 5 ml. of 5N acetic acid in 25 ml. stoppered conical flasks. The absorbance of the clear supernatant solutions were measured and the sample reading adjusted the blank. The amount of serpentine from the calibration curve was read.

Experimental and Discussion

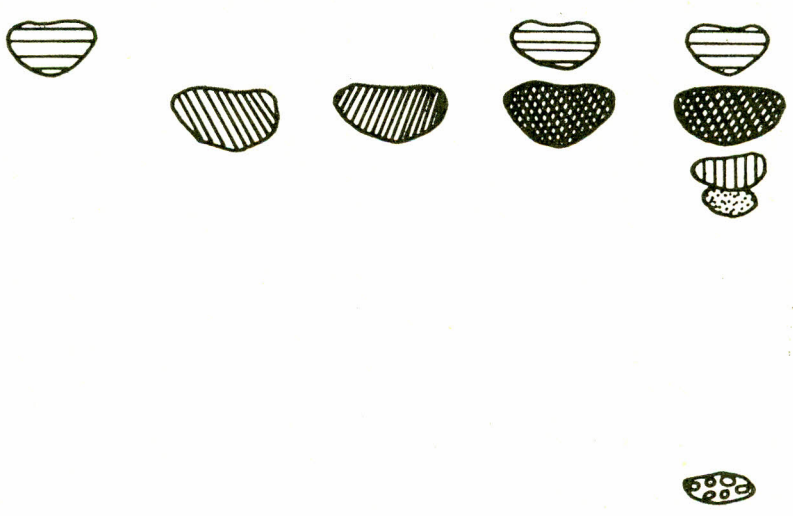
From the resolved electrophoretic pattern of serpajmaline (Fig. 1) it is seen that ajmaline and serpentine migrate to the same spot. It is, therefore, essential to measure the interference due to the presence of ajmaline on the UV absorbance of serpentine at 307 mμ.

The absorbance of 10, 20, 50 and 100 μg of ajmaline was measured in 10 ml. of 5N acetic acid (Fig. 2, curves 1,2,3,4 respectively). The absorbance due to 10 and 20 μg of ajmaline is not appreciable and concentrations such as these will not introduce a significant error in the estimation of serpentine (Fig 2).

Curves C, B and A show the effect of 10, 20 and 50 μg. of ajmal ineon the UV absorbance of 50 μg of serpentine. It can be seen that the increase in absorbance with 10 and 20 μg of ajmaline is not appreciable.

It should be pointed out, however, that the amount of ajmaline in the standard sample of serpajmaline, seldom exceeds 10 μg and is usually less than 10 μg. Consequently, while assaying serpajmaline there is hardly any appreciable interference from the presence of ajmaline.

Nevertheless, even when amounts larger than 20 μg of ajmaline are present, the method can still be applied effectively. First the contribution of different amounts of ajmaline at 307 mμ in 5N acetic acid is measured and subtracted from the total absorbance of the mixture of serpentine and ajmaline and then the amount of ajmaline present is estimated separately according to a method reported earlier.[5]



Serpentinine **Ajmaline** **Serpentine** **Mix of Serpentine Ajmaline and Serpentinine** **Serpajmaline**

Fig. 1.—Electrophoresis of Serpajmaline and its Constituent Alkaloids.

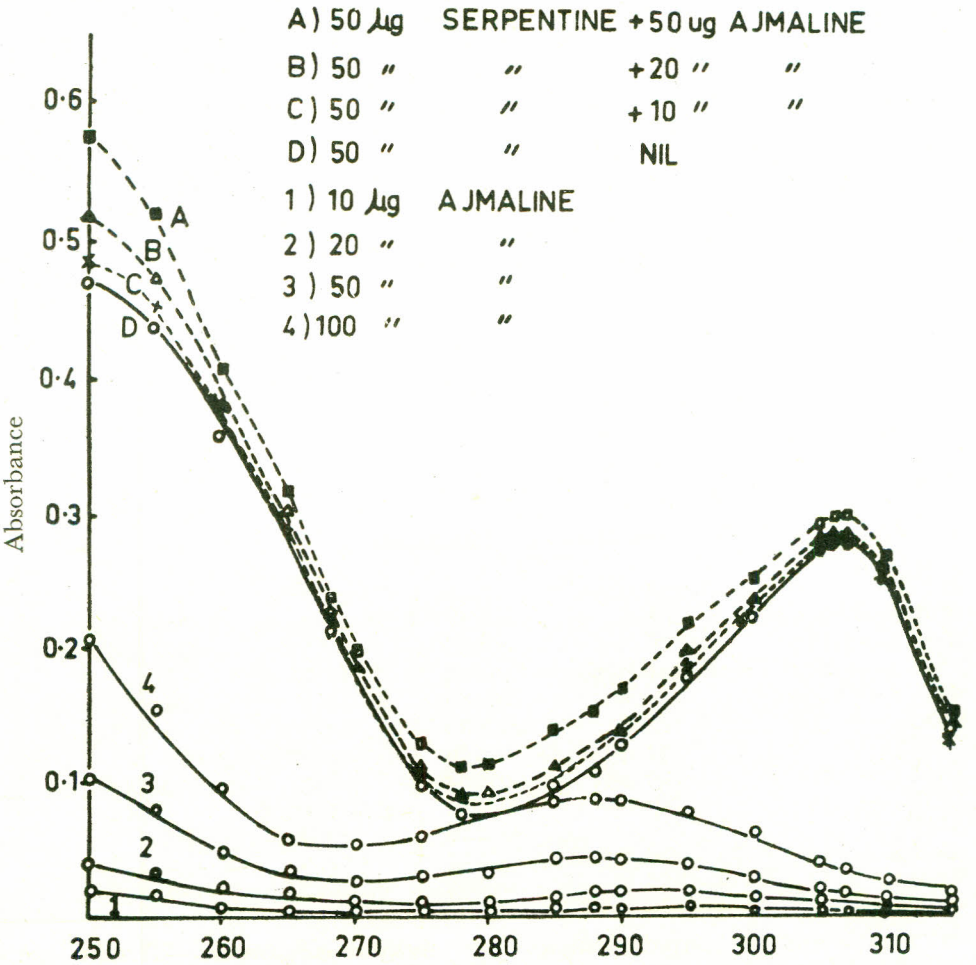


Fig. 2.—Showing effect of Ajmaline in mµ on UV curve of Serpentine.

The absorbance of 10, 20, 50, 100, 150 and 200 µg of ajmaline in 10 ml. of 5N acetic acid was measured at 307 mµ and the absorbance-concentration relationship upto 200 µg of ajmaline was found to be linear.

Table 1 shows the effect on the absorbance of serpentine by the addition of different amounts of

TABLE 1.—THE EFFECT OF THE ADDITION OF DIFFERENT AMOUNTS OF AJMALINE TO SERPENTINE ON THE SPECTROPHOTOMETRIC DETERMINATION OF THE LATTER.

Sr. No.	Ajmaline µg.	Ajmaline absorbance 307 mµ (a)	Serpentine +Ajmaline µg.	Absorbance of mixture (b)	Absorbance of Serpentine b—a	Serpentine found µg.	Difference µg.
1.	—	—	50+nil	0.285	0.285	50.5	+ .5
				0.280	0.280	50.0	0.0
				0.282	0.282	50.1	+0.1
2.	10	0.003	50+10	0.285	0.282	50.1	+0.1
3.	20	0.006	50+20	0.289	0.283	50.1	+0.1
4.	50	0.017	50+50	0.300	0.283	50.1	+0.1
5.	100	0.035	50+100	0.317	0.282	50.1	+0.1
6.	150	0.054	50+150	0.330	0.276	49.5	—0.5
7.	200	0.070	50+200	0.352	0.282	50.1	+0.1

Table 2.—Spectrophotometric Determination of Serpentine in Known and Unknown Samples.

Sr. No.	Samples of	Amount of sample per spot µg.	Absorbance at 307 mµ	Serpentine in 5 ml. solution µg.	Serpentine in the sample	Difference
	Known Composition					
1.	Serpentine .. 25.0 Serpentinine .. 25.0 Ajmaline .. 10.0		0.288	25.3	25.3 µg	+0.3
2.	Serpentine .. 24.20 Serpentinine .. 20.00 Ajmaline .. 20.14		0.276	24.6	24.6 µg	+0.4
	Unknown Composition					
3.	R.S.A.	.. 125.6	0.230	20.55	16.44%	
4.	M.B.S.	.. 108.8	0.120	10.7	9.8 %	
5.	Dajy — 187	.. 116.5	0.266	23.75	20.38%	
6.	SA_2 — 202	.. 104.325	0.215	19.4	18.39%	
7.	SA_2 — 195	.. 116.00	0.210	18.7	16.1 %	
8.	SSAP — 2	.. 108.81	0.286	25.55	23.5 %	
9.	SSAP — 1	.. 102.45	0.239	21.30	20.79%	
10.	SAIY — 201	.. 119.455	0.207	18.5	15.44%	
11.	SAIX — 201	.. 110.99	0.170	15.25	13.72%	
12.	SAS/231	.. 100.00	0.226	20.0	20.0 %	
13.	SA/231	.. 106.25	0.134	12.0	11.2 %	
14.	Se/231	.. 110.25	0.205	18.2	16.8 %	

ajmaline to serpentine and the corrected values obtained after subtracting the absorbance of ajmaline. The amount of serpentine present can be found from the calibration curve. The method is, therefore also applicable when larger amounts of ajmaline are present in combination with serpentine. Before assaying the unknown serpajmaline samples, mixtures, containing known amounts of ajmaline, serpentine and serpentinine were prepared so as to check the accuracy of the method.

Table 2 shows the amount of serpentine present in a number of samples of serpajmaline and the analysis of the known mixtures. From the accuracy obtained with the samples of known composition it can be inferred that the method can be successfully applied to the unknown mixtures.

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