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A METHOD OF EXTRACTION AND ESTIMATION OF AJMALINE IN DOG'S BLOOD

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Abstract. A rapid absorptiometric technique has been described for the extraction and estimation of microquantities of ajmaline in dog's blood. Ajmaline is extracted from serum with ethyl acetate and the red colour of nitroajmaline is measured spectrophotometrically at 510 nm. The method has an accuracy of $\pm 1.0\%$.

The Rauwolfia alkaloid ajmaline, first obtained in 1931–35,[1] was found to possess strong hypotensive properties. Although pharmaceutical investigations had been carried out by various workers for many years,[2,3] the alkaloid was first introduced by Kleinsorge[4,5] in 1958 for the therapy of cardaic arrhythmia and he was also the first worker to estimate small amounts of it in pharmaceutical products and body fluids. The procedure described by Kleinsorge and already referred to by us[6] is lengthy and time consuming. Its accuracy is $\pm 5.0\%$. In the course of pharmacological and metabolic studies of serpajmaline it was found necessary to develop a quick and accurate method for the estimation of ajmaline in blood in presence of serpentine, serpentinine and ajmalicine.

In the present paper a method has been described by which ajmaline has been estimated in dog's blood with an accuracy of $\pm 1.0\%$ by producing a fading red colour of nitroajmaline with concentrated nitric acid. This technique, the details of which have been published earlier,[7] has the advantage of being rapid; and its accuracy remains unaffected by the presence of interfering substances like serpentine, serpentinine and ajmalicine.[8]

Reagents

1. Ajmaline solution (0.1% solution of ajmaline in ethanol).
2. Ethyl acetate A.R.
3. Nitric acid (concd) A.R.

Apparatus

1. A Unicam SP 600 spectrophotometer with 1-cm cells.
2. Separating funnel, 100 ml.
3. Volumetric flask, 5 ml.
4. Centrifuge cones.

Experimental

1. *Extraction of Ajmaline from Synthetic Solutions.* Five ml deionised water is taken in a 100-ml separating funnel, to which is added 200 µl ajmaline (0.1% solution of ajmaline in ethanol). 2N NaOH, 0.2 ml or more, is added to adjust the pH to 10, followed by 20 ml ethyl acetate. The mixture is shaken vigorously and the upper layer is drained into a centrifuge cone and centrifuged for 5 min. The ethyl acetate layer gets completely separated and is transferred to a test-tube. The solution remaining in the separating funnel is again extracted with two aliquotes of 20 ml ethyl acetate as described above. The total extract of ethyl acetate is reduced in volume under vacuum and transferred to a 5-ml volumetric flask. The tube is washed completely with ethyl acetate and the volume is made exactly to 5 ml. One ml of this solution is transferred to a 10-ml cone and evaporated to dryness. The colour is developed by the addition of 5 ml HNO_3 (concd). Absorbance is read after 5 min. The results are given in Table 1.

The recovery is found to be 99.5–100% in simple extraction of ajmaline from synthetic solutions. This procedure was then applied to the extraction and determination of ajmaline in dog's blood. The effects of the pH, the type of solvent used for extraction, the nature of serum and the serum blank were studied. The extraction technique is the same as described above, the only difference being that 1 ml serum is diluted with 5 ml water before extraction.

2. *Effect of pH on the Recovery of Ajmaline from Dog's Serum.* Now 100 µg ajmaline was added to dog's blood serum and extracted as described in 1 above. The extract was evaporated and the volume made to 5 ml. The pH of this solution was 6, i.e. it was that of the serum. Two ml of this solution was then evaporated to dryness and again the colour was developed as in 1 above.

The pH value of the extract obtained as mentioned above was varied to 6, 9 and 10 before taking an aliquot of 2 ml for evaporation and colour development. The results are given in Table 2.

From Table 2 it is evident that the extraction of ajmaline was almost complete at a pH of 10 with an error of — 2.5%.

3. *Effect of the Nature of the Solvent Used for Extraction.* Kleinsorge extracted ajmaline with chloroform, but the recovery was low and also this solvent was comparatively more volatile than ethyl acetate used in the present method.

TABLE 1. EXTRACTION AND ESTIMATION OF AJMALINE IN SYNTHETIC SOLUTIONS.

Ajmaline added (μg)	Absorbance at 510 nm	Ajmaline found (μg)	Difference (μg)
40	0.495	40.0	—
40	0.490	35.5	—0.5
40	0.495	40.0	—

TABLE 2. EFFECT OF pH ON THE RECOVERY OF AJMALINE FROM DOG'S BLOOD.

pH	Absorbance at 510 nm	Amount of ajmaline added (μg)	Amount of ajmaline found (μg)	Difference (μg)
6	0·215	40	19	— 21·0
	0·22	40	20	— 20·0
	0·215	40	19	— 21·0
9	0·2	40	18	— 22·0
	0·21	40	18	— 22·0
	0·2	40	18	— 22·0
10	0·485	40	39	— 1·0
	0·48	40	39	— 1·0
	0·482	40	39	— 1·0

TABLE 3. COMPARISON OF THE EXTRACTIONS OF AJMALINE WITH CHLOROFORM AND ETHYL ACETATE.

	Absorbance at 510 nm	Amount present (μg)	Amount found (μg)	Difference (μg)
Ethyl acetate	1·3	100	100	—
	1·3	100	100	—
Chloroform	0·93	100	72	—28
	0·925	100	72	—28

As has been shown in the Table 3 ethyl acetate is a better solvent for the extraction of ajmaline than chloroform.

4. *Method of Extraction of Serum.* The accuracy of the results depends to a large extent on the method by which the serum is separated from blood. For best results it is necessary that the blood from which the serum is to be separated is allowed to stand for some-time before centrifuging. The buffy portion that appears on the top is removed with a glass rod and the blood is centrifuged again. It was found that freshly prepared serum gave the most accurate results.

The serum thus obtained was light yellow; pink or reddish sera were discarded.

5. *Determination of Blank.* The blank is determined by separating the serum as described above and carrying out all the operations as described in 1 except the addition of ajmaline solution. (Blank= Absorbance of 5 ml serum alone when extracted with ethyl acetate and treated as in 1 gave a blank value of 0.12).

On the basis of blank absorbance the recovery of ajmaline was studied by adding 62.5 μg of the alkaloid to the serum. This quantity was arbitrarily selected, as the recovery remained unaffected by blank above

TABLE 4. ESTIMATION OF AJMALINE (50–100 μg) ADDED TO THE BLOOD OF TWO DIFFERENT DOGS A AND B.

	Absorbance at 510 nm	Amount present (μg)	Amount found (μg)	Difference (μg)
Dog A	0·64	50·0	50·0	—
	0·65	50·0	50·0	—
Dog B	1·33	100·0	100·0	—
	1·29	100·0	99·0	—1

TABLE 5. RECOVERY OF AMOUNTS OF AJMALINE FROM 60 TO 400 μg IN 1 ml SERUM.

Amount of ajmaline	Absorbance at 510 nm	Amount present (μg)	Amount found (μg)	Difference (μg)
400 μg ajmaline extracted in 2 ml and 0.5 ml for absorbance (duplicate)	1·33	100	100	—
	1·31	100	100	—
	1·28	100	99·0	—1
	1·29	100	99·0	—1
100 μg ajmaline extracted in 1 ml. 0.5 ml for absorbance (duplicate)	0·65	50·0	50·0	—
80 and 60 μg ajmaline extracted in 2 ml and total solution taken for absorbance	1·04	80·0	80·0	—
	0·745*	60·0	59	—1
	0·785*	60·0	61	+1

*Two experiments were performed with 60 μg solution.

TABLE 6. AMOUNTS OF AJMALINE FROM 5 TO 50 μg IN 5 ml SERUM (blank of 5 ml serum = 0.25 at 510 nm).

Total absorbance 510 nm (serum)	Absorbance of ajmaline	Amount present (μg)	Amount found (μg)	Difference (μg)
0·285–0·25	0·035	5·0	5·0	—
0·35 –0·25	0·1	10·0	10·0	—
0·56 –0·25	0·31	25·0	26·0	+1
0·9 –0·25	0·65	50·0	51·0	+1

50 μg. In the present experiments the recovery was of the order of 99.2%.

Table 4 shows that the change of the donor of blood has no effect on the method of ajmaline estimation.

6. *Effect of the Blank Value in Relation to the Amount of Ajmaline Present in the Blood.* When the amounts of ajmaline in blood are above 80 μg the total extract after evaporation is still 2 ml as before, but for absorbance studies only 0.5 ml or 1 ml extract is evaporated to dryness. Here a blank on 0.5 ml and 1 ml serum extract, having an absorbance of 0.025 and 0.05 only, did not materially interfere in the results. With smaller amounts of ajmaline, however, the serum blank interferred in the absorbance measurements because for the estimation of small amounts of ajmaline the amount of serum has to be increased in order that the amount of ajmaline is between 10–50 μg in the extract (Table 6). It is, therefore, necessary to deduct the blank of the serum from the total absorbance for finding the correct absorbance of the ajmaline extracted. It was not possible to reduce the blank absorbance any further than 0.25.

In order to reduce the blank an attempt was made to remove the fat from serum. The procedure adopted was as follows: deionised water is added to the serum, followed by two fractions (10 ml each) of petroleum ether; a jelly-like film is formed between the serum and the water layers. Since it is very difficult to remove this layer it was filtered (with sintered glass funnel) and the tube and the funnel both were washed with ethanol. This alcohol was evaporated to dryness. Blank of 10 ml serum at 0.6 was reduced to 0.3. Now 100 μg ajmaline was added to the serum and extracted as before. It was found that in two experiments the recovery was very low and in one experiment only it was 80 μg. The reason for the low recovery is that some ajmaline remains behind in the jell and cannot be extracted with alcohol; this is confirmed by the presence of ajmaline in the petroleum ether fractions.

Discussion

The results are fully reproducible if care is taken to follow the experimental procedure described here before separating the ethyl acetate layer. It is necessary to centrifuge thoroughly for complete separation of the two layers of the solvent and the serum. Ethyl acetate should be transferred from one tube to another by means of a fine capillary-dropper without any leakage. Serum for analysis should be very carefully separated. This will keep the blank low at the level described in the text.

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