A SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF RESERPINE

By B. C. Bose and R. Vijayvargiya

From the Department of Pharmacology, M.G.M. Medical College, Indore, India

Received April 20, 1959

A spectrophotometric method for the estimation of reserpine in crude drugs and pharmaceutical preparations has been described. The method is capable of detecting microquantities of reserpine (0·25 μg./ml.). It is capable of estimating reserpine in crude drugs, alkaloidal mixtures, reserpine and serpentina tablets. The reserpine content in samples of Dehradun and Bengal varieties of *R. serpentina* has been found to be 0·117 and 0·103 per cent respectively.

A number of methods have been described for the assay of reserpine in pharmaceutical preparations[1–4], but not all are suitable for the estimation of reserpine in crude drug.

Many of the alkaloids present in rauwolfia plants have been grouped as feebly basic and strongly basic. Reserpine can be separated from more polar alkaloids by extraction from acidic solutions with chloroform, and in the present investigation an attempt has been made to estimate reserpine in crude drugs and alkaloidal mixtures by using this property.

As very few methods are available for the estimation of reserpine in natural preparations, it was considered advisable to develop a spectrophotometric method suitable for the micro-determination of this alkaloid. The method is based on the selective solvent extraction of reserpine from other alkaloids and its estimation in the ultra-violet region at 268 mμ. The method has been found to be simple and accurate in the present study.

Material and Method

The present work comprises two parts: the suitability of the spectrophotometric method for the estimation of reserpine in various solutions, and its application to the estimation of reserpine in crude drugs and pharmaceutical preparations.

A Beckman Spectrophotometer Model DU with 1 cm. standard silica cells was used and the absorbance maximum of reserpine in chloroform solvent found to be at 268 mμ. The relation between the concentration of reserpine and light absorption was established according to the following procedure.

Ten ml. of chloroform solution (containing 1 mg. of reserpine) was warmed with 10 ml. of 0·05N sulphuric acid with constant stirring to remove the chloroform. The acid solution was re-extracted with chloroform, the volume made up to 100 ml. and light absorption at various concentrations measured. The observations in Table I show a linear relation.

To remove polar and other interfering alkaloids and basic impurities from alkaloidal mixtures and crude drugs and to prepare a standard curve, the following procedure was adopted.

TABLE I

RELATION BETWEEN CONCENTRATION OF RESERPINE AND LIGHT ABSORPTION AT 268 Mμ WITH SLIT WIDTH AT 0·94 MM.

Conc. μg./ml.	0·25	0·50	1·00	2·00	4·00	6·00	8·00	10.00
Optical density	0·006	0·013	0·022	0·051	0·097	0·155	0·194	0·260

Aliquots, containing 1 mg. of reserpine in chloroform, were extracted with 4 × 10 ml. of 0·1N HCl, shaking every time for 2–3 minutes. The combined acid extract was treated with 5 ml. of chloroform, adding the wash to the main reserpine solution. After treating with 10 ml. of 0·05N H_2SO_4, chloroform was removed on a water bath as previously. The acid solution was quantitatively transferred to the separating funnel and extracted thrice with 10 ml. portions of benzene, shaking vigorously every time for 10 minutes. The combined benzene extract was then washed with 5 ml. of 0·05N H_2SO_4 adding the wash to the main acid solution. It was then extracted with 6 × 10 ml. of chloroform. The combined chloroform extract was washed with 5 ml. of 0·1N HCl and evaporated to dryness in a porcelain dish to remove traces of benzene. The residue was redissolved in chloroform and the reserpine content estimated at different concentrations. The findings are shown in Table II from which it may be seen that the relation is again linear.

TABLE II

RELATION BETWEEN CONCENTRATION OF RESERPINE IN μG./ML. AND LIGHT ABSORPTION AT 268 Mμ, WITH SLIT WIDTH 0·94 MM., AFTER TREATMENT WITH HYDROCHLORIC ACID AND BENZENE

Conc. μg./ml.	0·25	0·50	1·00	2·00	4·00	6·00	8·00	10·00
Optical density	0·004	0·010	0·020	0·041	0·088	0·137	0·187	0·252

On an analysis of data presented in Tables I and II, it will be seen that hydrochloric acid extraction did not result in any significant loss of reserpine in as much as only 2·5 per cent deviation in the recovery of reserpine (up to 1 mg.) with 4 × 10 ml. of hydrochloric acid was observed. Also, extraction with benzene did not interfere with the reserpine estimation provided that the solvent was completely removed from the extracts. Neither hydrochloric acid nor benzene thus showed the selective solvent property of chloroform which was found to be the most suitable solvent for the removal of reserpine from acid extracts.

After ensuring the suitability of the above method for the accurate estimation of microquantities of reserpine, it was considered necessary to determine the sensitivity of the test at different concentrations of the alkaloid and establish its optimal range. The estimations were made in graded concentrations of 0·5 to 4 mg. of reserpine and its recovery limits determined spectrophotometrically (Table III).

TABLE III

RECOVERY, PER CENT, OF KNOWN QUANTITIES OF RESERPINE AFTER HYDROCHLORIC ACID, SULPHURIC ACID AND BENZENE TREATMENT

Reserpine content mg.	Reserpine detected mg.	Deviation per cent
0·5	0·500	—
1·0	0·975	− 2·5
2·0	1·900	− 5·0
3·0	1·500	−50·0
4·0	1·700	−57·0

A reference to the figures in Table II and III indicates that the method is suitable for estimating reserpine in a range of 0·025 to 1 mg., beyond which the method becomes insensitive.

Estimation of Reserpine from known Alkaloidal Mixtures

After determining the suitability of the method for the estimation of known quantities of pure reserpine, the study was extended to the estimation of this alkaloid in the presence of other alkaloids of *R. serpentina*. Solutions of known quantities of reserpine, rescinnamine, serpentine, ajmaline, yohimbine and methyl reserpate were prepared in chloroform and treated with hydrochloric acid, sulphuric acid and benzene to find out whether the presence of other alkaloids interferred with the estimation of reserpine. The findings are shown in Tables IV and V.

TABLE IV

SHOWING PERCENTAGE RECOVERY OF RESERPINE FROM SOLUTIONS CONTAINING RESCINNAMINE AND RESERPINE

Sample No.	Reserpine μg.	Rescinnamine μg.	Reserpine recovered μg.	Deviation per cent
1	400	100	400	—
2	400	100	410	+ 2·5
3	400	200	410	+ 2·5
4	400	200	415	+ 3·7
5	400	350	414	+ 3·5
6	400	500	450	+12·5

TABLE V

SHOWING PERCENTAGE RECOVERY OF RESERPINE FROM ALKALOIDAL MIXTURES CONTAINING RESERPINE, AJMALINE, SERPENTINE, YOHIMBINE AND METHYL RESERPATE

Mixture No.	Reserpine μg.	Other alkaloids μg.	Reserpine recovered μg.	Deviation per cent
1	400	200	410	+2·5
2	400	200	410	+2·5
3	400	400	415	+3·7
4	400	400	410	+2·5

From Tables IV and V it is evident that common alkaloids present in *R. serpentina* in usual quantities, did not interfere in the assay of reserpine to any appreciable extent, excepting rescinnamine which interferes with reserpine estimation beyond a concentration of 350 μg. Since such a high concentration of rescinnamine is not present in the plant and

pharmaceutical preparations, this was not likely to affect the accuracy of the method. It would thus appear that rescinnamine is of limited solubility in benzene and the samples should be very thoroughly shaken to eliminate it completely.

Estimation of Reserpine in Tablets

Commercial samples of ten reserpine tablets were weighed and powdered and known quantities extracted with chloroform and filtered. The aliquots were analysed for reserpine content. The results are given in Table VI.

TABLE VI

RESERPINE CONTENT OF COMMERCIAL TABLETS

Sample No.	Reserpine content as labelled mg.	Reserpine recovered mg.	Deviation per cent
1	0·1	0·98	−2·0
2	0·1	0·096	−4·0
3	0·1	0·103	+3·0
4	0·1	0·102	+2·0
5	0·1	0·105	+5·0

From the above observations it is evident that colouring and other binding materials, present in the tablets did not interfere with the analysis of reserpine and it could be estimated with an accuracy of ± 4 to 5 per cent.

Estimation of Reserpine in Crude Drug

Though reserpine has now been established as the major alkaloid of *R. serpentina*, the plant contains a large number of other alkaloids which vary from species to species (Chatterjee[5,6]). There is thus considerable variation in the major and minor alkaloidal content in different varieties of *R. serpentina* growing in different regions of India. According to Hoffman[7] reserpine content in *R. serpentina* is about 0·14 per cent.

In the present investigation, the reserpine content in different samples of roots of *R. serpentina* has been analysed spectrophotometrically. Two important varieties of *R. serpentina*, collected from Dehradun and Bengal were selected for the present investigation.

TABLE VII

THE PERCENTAGE OF RESERPINE IN SAMPLES OF DEHRADUN AND BENGAL VARIETIES OF *R. serpentina*. THE RESULTS ARE THE AVERAGES OF FIVE SETS OF EXPERIMENTS IN EACH CASE

Variety of *R. serpentina*	Batch No.	Reserpine content per cent	Mean per cent
Dehradun	I	0·125	0·117
,, ...	II	0·110	
Bengal	I	0·110	0·103
,, ...	II	0·097	
Crude drug tablets	I	0·120	
,, ...	II	0·120	0·126
,, ...	III	0·140	

One gram of powdered root was extracted with chloroform in a micro-soxhlet apparatus for four hours, filtered and the volume made to 50 ml. An aliquot of 10 ml. was analysed for reserpine content as detailed in the section of preparation of standard curve. The findings are shown in Table VII.

As the reserpine content in different varieties of *R. serpentina* has not been definitely estimated, for comparison for the above figures, it was considered advisable to add a known quantity of reserpine to the original chloroform extract of the crude drug to find out whether the results of analysis in these circumstances would compare with the combined figures of reserpine found in the plant and the known quantity added to it. The results are shown in Table VIII.

TABLE VIII

SHOWING PERCENTAGE RECOVERY OF RESERPINE PRESENT IN CRUDE DRUG EXTRACTS TO WHICH ADDITIONAL QUANTITIES OF THE ALKALOID WERE ADDED.

Extract No.	Quantity of reserpine μg.	Quantity of additional reserpine added μg.	Total quantity of reserpine found μg.	Deviation per cent
1	250	200	470·0	+4·4
2	250	400	670·0	+3·1
3	200	200	412·0	+3·0
4	200	400	625·0	+4·1

From the above figures, it will be observed that the method has been found to be sufficiently accurate to measure the correct quantities of reserpine in these experiments with an accuracy of 3 to 4 per cent.

DISCUSSION

In the present investigation, reserpine content in roots of *R. serpentina* and other pharmaceutical preparations, has been analysed spectrophotometrically. In the first stage of this method, hydrochloric acid extraction of the chloroform extract did not cause any loss of reserpine but helped to removing interfering alkaloids, like ajmaline, serpentine, yohimbine and reserpic acid. Reserpine, being a feebly basic alkaloid could be easily taken up in chloroform from sulphuric acid solution in which it has been found to be soluble to a limited extent (1 mg./10 ml.). Extraction with benzene did not affect the content of reserpine in samples though it removed rescinnamine, colouring and other interferring basic impurities.

The method is applicable to samples containing micro-quantities of reserpine (0·25 μg./ml.) and has an added advantage that rescinnamine does not interfere with the assay (up to 350 μg./10 ml.). It has been observed that the method is suitable for analysis of crude drugs in which reserpine is present along with other alkaloids. The average reserpine content in samples of *R. serpentina* (Dehradun and Bengal varieties) has been found to be 0·117 and 0·103 per cent respectively.

Acknowledgments. Our thanks are due to Indian Council of Medical Research for sanction of a research grant for this investigation. We are grateful to Ciba and Co. for the liberal supply of pure samples of rauwolfia alkaloids.

References

1. Banes, Carol and Wolff, *J. Amer. pharm. Ass. Sci. Ed.*, 1955, **44**, 640.
2. Booth, *ibid.*, 1955, **44**, 568.
3. Dechene, *ibid.*, 1955, **44**, 657.
4. Kidd and Scott, *J. Pharm. Pharmacol.*, 1957, **9**, 176.
5. Chatterjee, *Fortschritte. d. Chemie Organ. Naturstoffe.*, 1953, 390.
6. Chatterjee and Talpatra, *Nature Wissenschaften*, 1955, **42**, 182.
7. Hofmann, *Helv. chim. acta*, 1954, **37**, 314.