COLORIMETRIC DETERMINATION OF RESERPINE IN POLYHERBAL FORMULATIONS

MD. AFZAL AZAN, A, RAHAMATHULLAH, P.JAYA CHANDRA REDDY and RAJEEV DUBE
J.S.S College of Pharmacy, Ootacamund – 643 001, Tamil Nadu

ABSTRACT: A simple spectrophotometric method is reported here for the estimation of reserpine in polyherbal formulations. Estimation is based on the reaction with 3-methylbenzolinone-2-hydrazone (MBTH) reagent in presence of cerric ammonium sulphate to yield a violet coloured chromogen, which exhibits and absorption maxima at 580 nm. The chromogen is stable for 10 minutes.

INTRODUCTION

Rauwolfia is an important herbal drug, indigenous to India which has been used from ancient times for treatment of a variety of disease. The active principle reserpine is used in the modern medicine for the treatment of hypertension and as a tranquilizing agent. Herbal formulations containing rauwolfia powder or dried aqueous extract of the root, manufactured by different pharmaceutical companies are indicated particularly in mental disorders.

Reserpine is its pure form or from crude rauwolfia powder or from dried extract of rauwolfia can be estimated by gravimetric, spectrophotometric, colorimetric and fluorimetric methods. Literature survey revealed that no colorimetric method for estimation of reserpine is reported for polyherbal formulations. We report here a simple colorimetric method which is easy to perform and has high sensitivity and selectivity. The present method of estimation utilizes the reaction of reserpine with MBTH reagent in presence of cerric ammonium sulphate to form a violet coloured chromogen with an absorption maxima at 580 nm.

EXPERIMENTAL

Instrument:
Shimadzu UV 160A Spectrophotometer

Reagents:

(a) 2-Methylbenzthiazolinone-2-hydrazone (MBTH) reagent 0.2% w/v

(b) Cerric ammonium sulphate solution 0.5% w/v

(c) Sulphuric acid 10% v/v

(d) Glacial acetic acid 6% v/v

(e) Alcohol 9% v/v

Standard Solution

A solution of reserpine I.P at a concentration of 100 µg/ml in 6% acetic acid.

Estimation
Five polyherbal products containing reserpine were selected. Twenty tablets of each product were crushed into powder and a weight of the powder, equivalent to 2.5g of powdered rauwolfia serpentine was extracted with 9% alcohol in a soxhlet apparatus for 4 hr. all the solutions were protected from light throughout the experiment. Extract was washed into a 100 ml volumetric flask with alcohol, cooled, diluted with alcohol to volume and mixed 20ml was transferred to a 50 ml beaker, and evaporated to 2ml on a steam bath. 5ml of 6% acetic acid was added and the mixture was heated to expel remaining alcohol. The solution was cooled, transferred to a 10 ml volumetric flask and are diluted to volume with 6% acetic acid. A 5 ml aliquot was transferred to a 10x 75 mm test tube. 1 ml of MBTH reagent and 0.5 ml of cerric ammonium sulphate were added and diluted to volume wit 10% v/v sulphuric acid. After 5 minutes absorbance as measured at 580 nm against a reagent black and concentration was calculated from the standard curve (Fig 1) prepared with similarly treated known samples (5-50 µg/ml) of reserpine. Results are given in table 1.

Reserpine content of all the formulations (T1-T5) was also determined by using standard method5 (Table 1).

RESULTS AND DISCUSSION

Replicate analysis of each sample containing a composite of 20 table were carried out the violet coloured chromogen formed was stable for 10 minutes. The calibration curve was linear and obeyed beer’s law in the concentration range of 5-50 µg/ml. The recovery data were determined by adding two different amount of reference standards to the preanalysed polyherbal formulations. The proposed method produced satisfactory recovery for all the formulations (T1-T5).

The results obtained from the proposed MBTH method is comparable to standard method and indicate good correlation. The assay described is precise and reproducible for the quantification of reserpine in polyherbal formulation.

Table -1

| Formulations | Label claim crude drug /tab (mg) | Total reserpine by official method µg/tab | Total reserpine by proposed method µg/tab | Deviation (±) | % Recovered by proposed method |
|--------------|----------------------------------|----------------------------------------|-----------------------------------------|--------------|----------------------------------|
| T1           | 70                               | 70.14                                  | 70.25                                   | 0.11         | 99.68                            |
| T2           | 20                               | 40.50                                  | 41.25                                   | 0.25         | 99.85                            |
| T3           | 20                               | 41.03                                  | 41.50                                   | 0.47         | 98.60                            |
| T4           | 325                              | 182.21                                 | 182.65                                  | 0.44         | 98.62                            |
| T5           | 20                               | 78.93                                  | 78.75                                   | 0.18         | 99.45                            |

* Saragandha extract
Acknowledgement

The authors are grateful to the college authorities for providing facilities to carry out this work.

REFERENCES:

1. The Indian Pharmaceutical Codex, Vol. I, Indigenous Drugs, council of scientific and industrial research, New Delhi (1953)

2. British Pharmaceutical Codex 1954, pharmaceutical press, London (1954)

3. Pillay, P.P., S.B Rao and D.S Rao Indian. J Pharm., 17, 95 (1955).

4. Me Murlen, W.H., Pazdera, H.J., Missan, S.R., Cicio, L.L. and Grenfell T.C J Am Pharm Assoc 44,446 (1955).

5. The United states Pharmacopoeia, 23,1366 (1955).

6. Wunderlich, H., Pharm. Zentralhalle, 96,68 (1957).

7. Eder, R. and ruckstuhl, O., Pharm Acta Helv., 18,396 (1943).

8. Safarik, L and Spinkova, V., Cesk. Slov farm., 7,76 (1958)