SHORT COMMUNICATION

COLORIMETRIC DETERMINATION OF PIPERAZINE

P. K. SASI AND R. KALEYSA RAJ

Department of Biochemistry,
University of Kerala, Trivandrum

Summary: A sensitive method for the quantitative determination of piperazine is described. The method is precise and responds linearly from 25 μg to 500 μg and above of the material. The procedure is based on the formation of a complex of piperazine with reineckate in neutral or acid medium. The complex can be separated by centrifugation. It is then dissolved in acetone and estimated at 530 nm in a colorimeter. Piperazine present in trichloroacetic acid extractrs of biological samples can also be estimated by this method.

Key words: Piperazine, reineckate, serum, urine, liver, human, human *Ascaris lumbricoides*

INTRODUCTION

A sensitive method for the quantitative estimation of piperazine is presented in this paper. It is based on the formation of a complex of piperazine with reineckate in neutral or acid medium. The method is a definite improvement over the existing methods for the estimation of piperazine (1,3). Piperazine present in biological samples can also be estimated by this method. Recoveries in this method range from 95 to 100% of the applied sample in serum, urine and liver homogenate.

MATERIALS AND METHODS

Piperazine salts such as phosphate, adipate and citrate were obtained from Indian drugs and Pharmaceuticals Ltd. Ammonium reineckate was of BDH grade. Other materials such as acetone and tricholoracetic acid were of analar grade. For the assay, 0.5 *ml* of a 3% solution of ammonium reineckate is added to a series of tubes containing 1 *ml* of a neutralised solution of piperazine ranging from 25 μg to 500 μg. After mixing, the tubes were kept in ice for 3 *hr*. These were then centrifuged for 10 min at 2000 *rpm* and washed twice, each time with 1 *ml* of ice-cold water. The supernatants were discarded. After the second washing, the tubes were kept inverted for about 10 min to drain off the water completely. The residues were then dissolved in 1 *ml* of acetone and the optical density read at 530 nm (vide Fig. 1). The use of the method in biological systems were tested using serum, urine isolated liver and intact human *Ascaris lumbricoides*. Serum and urine samples were mixed with varying concentrations of piperazine and incubated at 37° for 1 hr. These were then deproteinized with equal volumes of 10% TCA solution. Aliquots of TCA extracts were used for the assay. The liver was homogenized and the homogenate incubated with varying concentrations of piperazine for 1 hr. It was then deproteinized with 10% TCA and the filtrate was assayed. The adult round worms were incubated for 6 *hr*

in modified Tyrode solution containing piperazine (2). The worms were then washed with water and the muscle collected and worked up as in the case of isolated liver.

RESULTS

Fig. 1 shows the absorption pattern of piperazine reineckate complex and that of pure reineckate in acetone solution. There was excellent proportionality between concentration and optical density, as shown by the solid line in Fig. 2. Piperazine salts such as the phosphate, adipate and citrate behaved similarly in the assay. Recovery of added piperazine from serum, urine and liver homogenate is also shown in Fig. 2. The result from the human round worm is discussed.

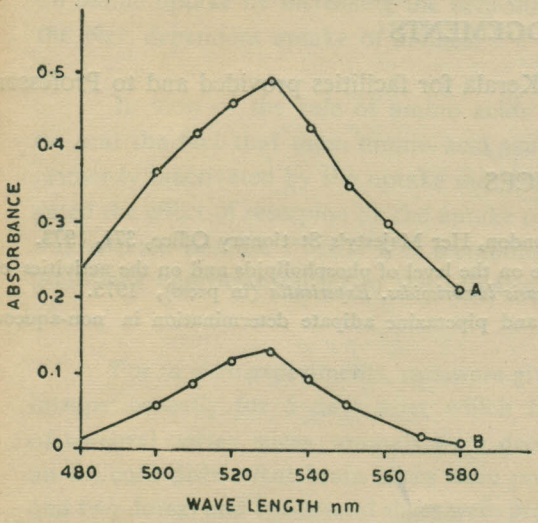

Fig. 1: Absorbance of Piperazine-reineckate complex and reineckate at different wave lengths.

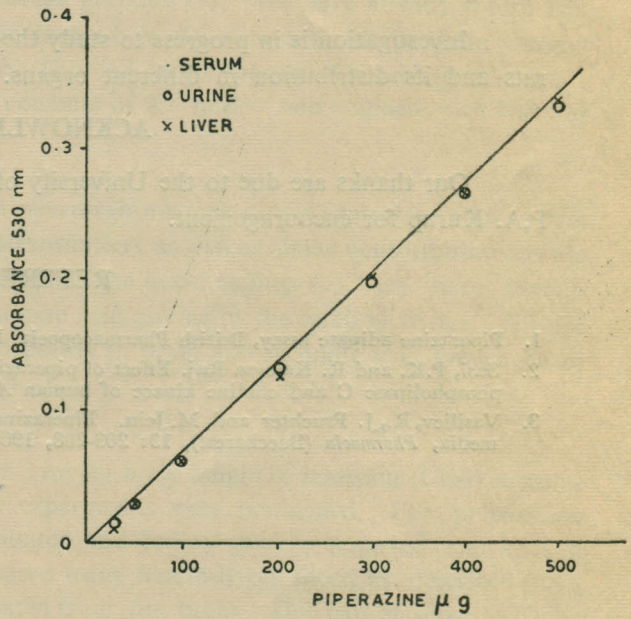

Fig. 2: Recovery of known quantity of Piperazine added to serum, urine and liver homogenate. The values shown represent Piperazine recovered and assayed as described. Based upon absorbance produced by the standards the theoretical recovery values are indicated by the solid line.

DISCUSSION

The absorption peak of both reineckate as well as the complex was at 530 nm showing that it was due to the reineckate moiety. Hence all the readings were taken at 530 nm. Alkaline medium was found unsuitable for the precipitation of the complex. But neutral and acid media were suitable and gave similar results. This method has a sensitivity from as low a concentration as 25 μg to high concentrations. Piperazine free blanks in all the cases were free of any colour. Hence materials such as choline, which produce a positive reaction with reineckate are present in negligible amounts to cause any error in the assay.

The result with the nematode was interesting, Both under *in vitro* and *in vivo* conditions, high concentrations of piperazine are needed to cause paralysis of this parasite. But surprisingly lower concentrations were shown to produce effects on the activities of enzymes of phospholipid metabolism (2). The results indicate that the biochemical changes brought about in the worm may be proportional to the amount of piperazine accumulating in the muscle of the parasite. This aspect is currently under detailed investigation. A number of determinations carried out with one concentration of piperazine gave the following results. Quantity of piperazine gave the following results. Quantity of piperazine in external medium was 200 *mg*/worm of average weight 2 *g*, while the quantity present in the muscle tissue after 6 hr incubation, was 12 *mg* ±2.1 (SE) for 7 determinations. The latter concentration can be said to be effective in homogenate systems.

Investigation is in progress to study the duration of persistence of the drug in laboratory rats and its distribution in different organs.

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