that of leaf extract suggesting the possible use of seed coat extract for further evaluation and purification in search of active principle.

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Differential Pulse Polarographic Determination of 1,4-Benzodiazepine Psychotropic Drugs in Pharmaceutical Formulations and urine Samples

P. R. K. REDDY, N. Y. SREEDHAR AND S. J. REDDY

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502

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A simple, rapid, and sensitive differential pulse polarographic method is developed for the determination of 1,4-benzodiazepine psychotropic drugs such as pinazepam, prazepam and temazepam in pharmaceutical formulations and urine samples, by the aid of universal buffers of pH 2.0 to 12.0. The developed procedure has been applied for the determination of these drugs in pharmaceutical formulations and urine samples as well as simultaneous determination in a single run.

SINCE the introduction of chlordiazepoxide hydrochloride in 1960, a large number of 1,4-benzodiazepine compounds have been investigated as tranquilizers, hypnotics, sedatives and antidepressants[1]. The 1,4-benzodiazepines are usually present in trace amounts following therapeutic administration because they undergo extensive biotransformation and tissue distribution. Pinazepam [7-chloro-1,3-dihydro-5-phenyl-1- (prop-2-ynyl)-2H-1, 4-benzodizepin-2-one], prazepam [7-chloro-1-cyclopropylmethyl)-1,3-dihydro-5-phenyl-2H-1, 4-benzodiazepin-2-one] and temazepam [7-chloro-1,3-dihydro-5-phenyl-1-methyl-2H-3-hydroxy-1,4-benzodiazepin-2-one] all have more pronounced psychotropic actions than other benzodiazepines, and they are also used in small doses. Therefore, a reliable analytical method is needed for the accurate determination of these drugs. Several methods have been described for the determination of benzodiazepines in biological fluids which are based on electron capture gas liquid chromatography[2], luminescence determination on thin layer chromatographic plates[3], high pressure liquid chromatography[4] and spectrophotometry[5]. The aim of the present work is to study the differential pulse polarographic behaviour of the title compounds and use it for their determination in pharmaceutical formulations and urine samples.

The details of the equipment used for the present investigation, theoretical, experimental procedures of these techniques were discussed elsewhere[6]. The polarographic behaviour of pinazepam, prazepam and temazepam was examined over the pH range 2.0 to 12.0. All these

Table 1: Assay of Pinazepam, Prazepam and Temazepam at pH 4.0

Compound	Labelled amount (mg)	Amount* found (mg)	% Recovery	Standard deviation
Pinazepam formulation				
A	10	9.89	98.90	0.036
B	5	4.98	99.60	0.002
Prazepam formulation				
A	5	4.96	99.20	0.004
B	10	9.92	99.20	0.008
Temazepam formulation				
A	10	9.98	98.60	0.007
B	5	4.95	99.80	0.002

* Average of three determination

Table 2: Recovery of Pinazepam, Prazepam and Temazepam in urine samples at pH 4.0

Compound	Amount added (mg)	Amount found* (mg)	% Recovery	Standard deviation
Pinazepam	2.0	1.96	98.00	0.004
Prazepam	2.5	0.47	98.80	0.003
Temazepam	3.0	2.95	98.30	0.005

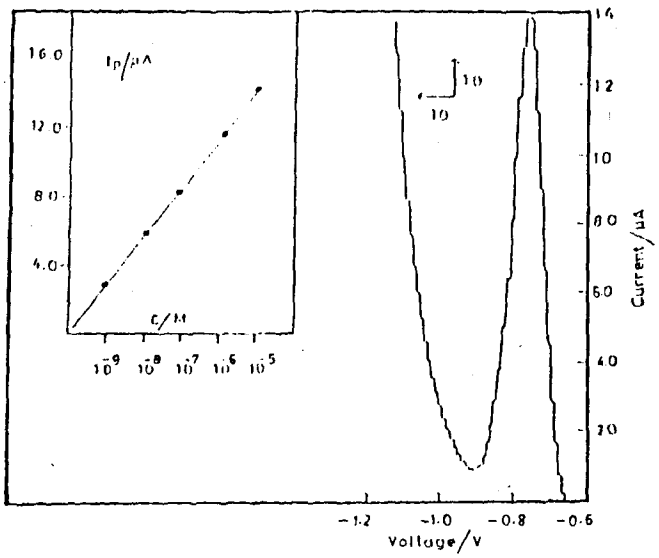
* Average of three determination

compounds were found to give a single well-defined peak in all the buffer systems studied. The single peak was attributed to the reduction of azomethine functional group to hydroxyl amine group in a two electron process[7]. The electrode processes for the drugs are found to be adsorption free and diffusion controlled which is evidenced from the linear plots of i_m versus $t^{2/3}$ (where i_m=maximum peak current and t=drop time) passing through the origin[8,9]. The reduction potential (E_m) values of the title compounds are found to depend on pH and shift towards more negative potentials with increase in pH of the buffer solutions[10].

The differential pulse peak obtained in the pH 4.0 is well resolved and may be utilised for the analysis of pharmaceutical preparations by employing both calibration and standard addition methods. The peak heights were found to be linear in the range from 1.5×10^{-5} to 1.4×10^{-9} M with lower detection limit of (ldl) 1.15×10^{-9} M for pinazepam, 1.25×10^{-5} to 1.5×10^{-9} M with ldl of 1.05×10^{-9} M for prazepam and 1.35×10^{-5} to 1.72×10^{-9} M with ldl of 1.25×10^{-9} M for temazepam. The ldl was calculated using the equation[11] dl=3xSd/m where Sd=standard deviation and m=slope of the calibration plot.

Standard solutions of the samples (1.0×10^{-5} M) were prepared in pure dimethylformamide (DMF). In polarographic cell, 1 ml of standard solution was transferred and mixed with 9 ml of the supporting electrolyte (pH 4.0)

Fig.1 : Typical differential pulse polarogram of pinazepam in urine at pH 4.0



Concentration = 1.0 x 10^{-5}M; Drop time = 2 Sec; Pulse amplitude = 60 mV. The linear plot of peak current versus concentration of pinazepam is shown in the inset

and deoxygenated with nitrogen gas for 5 min. After recording the polarogram, small increments (0.2 ml) of standard solutions were added and polarograms were recorded under similar conditions. The optimum conditions for the determination of these compounds in pH 4.0 was found to be a drop time of 2 s, a pulse amplitude of 60 mV and applied potential of -0.78 V, -1.02 V and -1.31 V for pinazepam, prazepam and temazepam respectively. The relative standard deviation and correlation coefficient were found to be 1.43% and 0.995 for pinazepam, 1.48% and 0.998 for prazepam and 1.32% and 0.991 for temazepam for 10 replicants. These drugs are available in tablet dosage forms. The assay results of these drugs were calculated by referring the calibration plot. The results of the dosage forms were furnished in Table 1.

A calibration plot for pinazepam was constructed in accordance with the limits at which the unchanged drug is excreted. Different amounts of pinazepam were added to a fixed volume of urine. Aliquots of these spiked urine samples were diluted with the supporting electrolyte and the polarograms were recorded. The calibration plots ranging 2.5 to 75 ng/ml of these drugs were determined in urine. The analysis of numerous samples indicates a relative standard deviation of 2.8% and 2.0% at lower and higher concentrations. Prazepam and temazepam were determined in the urine with the same concentration range and the results were reported in Table-2. Typical differential pulse polarogram of pinazepam in urine sample is shown in Figure 1.

Further the described procedure is selective for simultaneous determination of the above psychotropic drugs, because of wide separation in their peak potentials (≥240 mV). This method is sensitive enough to measure concentrations as low as those encountered after therapeutic dosage and does not require time consuming separation of ingredients. The described method found to be simple, rapid, sensitive and can be applied to routine usage.

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