2 h before injection[7]. Mean increase in paw volume was measured and percentage inhibition was calculated.

Qualitative chemical analysis revealed the presence of alkaloids, carbohydrates, proteins, saponins and tannins (Table 1). Antiinflammatory effect of extracts against carrageenan- induced inflammation is shown in Table 2. Comparatively the ethanolic extract showed significant antiinflammatory activity at 200 mg/kg dose ($P<0.01$) level, which was comparable with that of ibuprofen 100 mg/kg standard drug ($P<0.01$), where as the petroleum ether extract didn't show much significant activity when compared to standard. The percentage protection of the extracts is shown in the Table 3.

Inflammation is a response of the tissue to an infection, irritation or foreign substances. A variety of chemical agents like histamine (1 mg/ml), carrageenan (1% w/v), dextran (60 mg/ml), have been used to induce edema in the feet of rodents. Antiinflammatory activity of an extract can be determined by their ability to reduce or prevent oedema[8]. The development of carrageenan-induced edema is biphasic, the first phase is attributed to the release of histamine, 5-hydroxytryptamine and kinins, while the second phase is related to the release of prostaglandins[9-11]. The plant has direct or indirect action over this and that was the result of its antiinflammatory action.

The present study concludes that the plant *Nothapodytes foetida*, selected for antiinflammatory activity has shown appreciable results which supports the claim of local people and much work in this direction has to be done to confirm its utility in higher models.

REFERENCES

1. Joshi, R., Jain, N.K and Gar, B.D., **Indian Drugs,** 1978, 16, 18.
2. Dhar, M.L., Dhar, M.N., Dhawan, B.N., Mehrotra, B.N., Srimal, R.C. and Tandon, J.S., **Indian J. Exp. Biol.,** 1973, 11, 43.
3. Govindachari, T.R. and Viswanathan, N., **Phytochemistry,** 1972, 11, 3529.
4. Kokate, C.K., In ; Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, Delhi, 1994, 108.
5. Kulkarni, S.K., In; Hand book of Experimental Pharmacology, 3rd Edn., Vallabh Prakashan, Delhi, 1999, 128.
6. Winter, C.A., Risley, E.A. and Nuss, C.W., **Proc. Soc. Exp. Biol. Med.,** 1962, 111, 544.
7. Tonussi, C.R. and Ferreira, S.H., **Eur. J. Pharmacol.,** 1994, 251, 173.
8. Turner, R.A., In; Screening Methods in Pharmacology, Academic Press, London, 1965, 153.
9. Larsen, G.L. and Henson, P.M., **Ann. Rev. Immunol.,** 1983, 1, 335.
10. Brooks, P.M. and Day, R.O., **N. Engl. J. Med.,** 1991, 321, 1716.
11. Vane, J. and Booting, R., **FASEB J.,** 1987, 1, 89.

Reversed Phase HPLC Method for Determination of Glimepiride in Tablet Dosage Form

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Simple, rapid and precise reversed-phase HPLC method has been developed for the quantitation of glimepiride in tablet on a Hypersil C-18 (15 cmx3.9 mm) column using a mobile phase consisting acetonitrile:0.05 M monobasic potassium phosphate (pH 6.0) (40:60 v/v) at a flow rate of 1.5 ml/min and detection at 210 nm. The retention time of glimepiride have been found to be 7.8 min and recoveries were between 99-101%. Validation of the proposed method also been done.

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Glimepiride is a sulphonylurea class of antidiabetic agents, which stimulate insulin release. Its major site of activity is membrane receptor on pancreatic β-cells, where it acts via K_{ATP}-channels, resulting in membrane depolarisation and release of insulin. It is a relatively new sulphonylurea that is conveniently administered as a once daily dose in patients with type 2 diabetes mellitus not well controlled by diet alone. Moreover, with a favourable effect on the cardiovascular system, it offers advantages over conventional sulphonylurea to diabetic patients with ischemic heart disease. Glimepiride is not official in any pharmacopoeia. Literature survey revealed only one HPLC method[1] for its determination in human plasma and one UV-spectrophotometric method[2] for quantitation of tablet dosage form. No HPLC method has been reported for the quantitation of glimepiride in the formulations. The present work describes a simple, precise and accurate reversed-phase HPLC method for the estimation of glimepiride in tablet dosage forms.

All chemicals/solvents used were of AR/HPLC grade and standard glimepiride was provided by Sun Pharmaceutical Industries Ltd. Mumbai. A Shimadzu HPLC (LC-10AT VP) system was used for the analysis. The method was carried out on a Hypersil C-18 (15 cm×3.9 mm) column as a stationary phase and acetonitrile:0.05 M monobasic potassium phosphate (adjusted to pH 6.0 with trietylamine, 40:60 v/v) as the mobile phase at a flow rate of 1.5 ml/min. A rheodyne injector with a 20-µl loop was used for the injection of samples. Detection was done at 210 nm with sensitivity 0.010 AUFS. The mobile phase was filtered through a 0.45 µ membrane filter (Millipore) and degassed. The analysis was carried out at room temperature (about 20°).

Standard stock solution was prepared containing 1 mg/ml of glimepiride in methanol. Subsequently dilutions were made to get the concentration about 20 µg/ml in mobile phase. Sample solution was prepared in 50 ml volumetric flask by shaking tablet powder equivalent to 5 mg of glimepiride in methanol. This solution was filtered (Whatman No.1) and further dilution was made with mobile phase. A steady baseline was recorded with optimised chromatographic conditions. Chromatographs of standard solution (six replicates) and sample solution (two replicates of each) were recorded (one of which is depicted in fig.1). The retention time of glimepiride was found to be 7.8 min. The concentrations of glimepiride in sample solution were obtained by comparing with the standard solution.

Validation of the proposed method was demonstrated by various parameters[3,4]. Accuracy of the method was studied by recovery experiments. Reference standard drug at the level of 25, 50, 75 and 100% of the label claim were added to the tablet powder equivalent to 5 mg of glimepiride. These were analysed by injecting three replicate of each sample solution and the percent recovery were calculated. Precision of the method was demonstrated by reproducibility studies. This was done analysing six samples prepared from homogeneous sample. Specificity was carried out by exposing the samples to different stress conditions for 24 h such as acidic (0.1N HCl, 40°), basic (0.1N NaOH, 40°), oxidation (3% v/v H_2O_2, 40°), heat (60°), UV light (254 nm), and humidity (75% RH, 40°), before analysis by proposed method. Linearity and Range of the method was determined by analysing standard solutions containing 10 to 40 µg/ml (50 to 200% of targeted level of the assay concentration). The calibration curve was plotted using area under curve Vs concentration of the standard solution. Ruggedness of the method was evaluated by carrying out the experiment by different analysts and on different days. Stability of standard and sample solution was ascertained by analysing it periodically. Robustness of the method was demonstrated by variation in composition of mobile phase (±5%), concentration of buffer (±5%) and pH of buffer solution (±0.1).

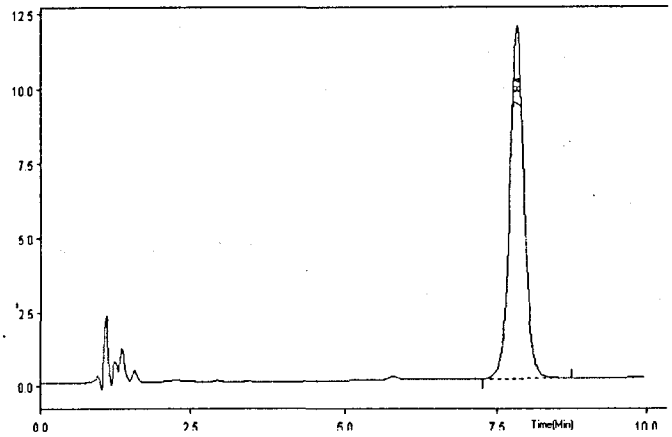

Fig. 1: Chromatogram of glimepiride

The chromatographic parameters were validated by system suitability studies[5] and peak asymmetry and col-

TABLE 1: SYSTEM SUITABILITY STUDIES

Parameters	Results*
Peak Area (% RSD)	194.5 (0.60)
Capacity factor	6.92
Tailing Factor	1.05
Theoretical Plates (per column)	7636

*Mean of six observations

TABLE 2: ANALYSIS OF FORMULATIONS AND RECOVERY STUDIES

Formulation (Tablet)	Label claim (mg/tab)	Estimated*		%RSD	Percent recovery** ± S. D.
		Amount (mg/tab) ± S. D.	Percent label claim		
Gepride (Medley)	1	1.02	102.0±0.22	0.26	100.0±0.13
	2	1.99	99.5±0.50	0.50	99.6±0.21
	4	3.98	99.5±0.10	0.10	99.9±0.19
Amaryl (Aventis)	1	1.01	101.0±0.02	0.02	100.8±0.06
	2	2.02	101.0±0.05	0.05	100.6±0.10
		Mean	100.6±0.19	0.19	100.2±0.14

*Mean of six observations, **Mean of three observations. Assay was studied by analysing six sample solutions (two replicate of each) prepared from homogenous sample. Accuracy of the method was studied by standard addition method and analysed by injecting three replicate of each sample solution.

umn efficiency were determined (Table 1). The precision data showed the reproducibility of the assay procedure as satisfactory and %RSD was found to be 0.19 (Table 2). Accuracy studies indicated recoveries of the drugs between 99-101% and the mean percent recovery of the added standard drug was found to be 100.2% (Table 2).The results of specificity studies indicated no interference from excipients, impurities and degradation products due to various stress conditions, and assured that the peak response was due to a single component only. The linear relationship was obtained in the concentration range 10-40 µg/ml with the equation 9.8411x-1.8657 and correlation coefficient 0.9993. Ruggedness study signified the reproducibility of the method under different conditions and %RSD was found to be 0.4586 and 0.9248 for different analysts and different days respectively. The method was found to be robust with respect to theoretical plates, retention time, etc. and %RSD of assay results was found to be not more than 0.98. Limit of detection and limit of quantitation was found to be 0.8 µg/ml and 2.5 µg/ml, respectively. The proposed HPLC method was found to be *simple, accurate, precise, linear, rugged and rapid.* Hence this method can be applied for the quality control of tablet formulation.

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REFERENCES

1. Lher, K.H. and Damm, P., **J. Chromatogr.,** 1990, 526, 497.
2. Altinoez, S. and Tekeli, D., **J. Pharm. Biomed. Anal.,** 2001, 24, 507.
3. *Validation of Analytical Procedures: Methodology,* ICH Harmonised Tripartite Guidelines, 1996.
4. Text on Validation of Analytical Procedures, ICH Harmonised Tripartite Guidelines, 1994.
5. United State Pharmacopoeia - XXIV, United State Pharmacopoeial Convention Inc., Rockville, 1998, 1923.