TABLE 1: ASSAY OF ROTACAP FORMULATION AND RECOVERY STUDIES.

Trial Bt. No.*	Assay		Recovery study			
	Label amt. μg/ rotacap	Amount found (20 rotacaps μg and %content)**	Amt. added (μg)	Total found μg**	Amount of standard recovered μg	% recovery
B- I	12	236 (98.3)	100	335	98.6	98.6
B-II	12	237.2 (98.8)	150	390	152.6	101.7

*The rotacaps of two separate batches obtained from the same manufacturer were analyzed. ** Average of 6 observations.

The Beer's law was obeyed in the concentration range of 1-40 μg/ml, molar absorptivity determined to be $2.26X10^{5}$ l/mole.cm and Sandell's sensitivity 0.0707 μg/cm^2-0.001 absorption units. The regression equation (Y=a+bx) was obtained by a linear least squares treatment of the results, established slope as 0.012686 and intercept 0.0672 with standard deviation of 0.16 and coefficient of variation 0.10. The data from recovery studies indicated no interference of excipients present in the formulation. The developed method was thus found to be sensitive, accurate, precise and reproducible and can be used for the routine determination in rotacap formulation.

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Concurrent Assay of Metformin and Glimepiride in Tablets Using RP-HPLC with Wavelength Programming

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A rapid assay procedure based on RP-HPLC has been developed for the simultaneous determination of metformin and glimepiride in dosage form. The HPLC determination was carried out on a μBondapak C_{18} (300x3.9 mm) 10 μm with use of a flow rate of 1.0 ml/min. The programming regime

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was, 0-5.8 min at 265 nm, 5.8-9.0 min at 230 nm and 9.0-11 min again at 265 nm. The calibration graphs were linear in the range of 400-600 and 1.6-2.4 μg/ml for metformin and glimepiride respectively with correlation coefficient of 0.9999 for both.

RP-HPLC method is the accepted method for the assay of drugs in the pharmacopoeias of developed countries[1]. The present paper involves assay of a combination of *metformin (500 mg) and glimepiride (2 mg) that is used* against type II diabetes. In the dosage form, metformin's dosage is 250 times higher than glimepiride because of which at balanced wavelength metformin's response goes out of scale. While using a wavelength programming it can be possible to bring response in quantifiable limit. In the present paper isocratic separation and simultaneous detection of the two drugs from the formulation using a wavelength programming is described.

Absence of suitable analytical method for assay of glimepiride in pharmaceutical dosage form, led us to develop a new method for its assay. For metformin there are several methods available in solution and in pharmaceutical preparation by HPLC and GC[2-5]. Spetrophotometric determinations also have been carried out for metformin[6]. There was no method has been reported for simultaneous estimation of metformin and glimepiride.

Stock Solution (1 mg/ml) of the individual drugs were prepared by dissolving 100 mg of each drug in 100 ml of methanol. Appropriate concentrations of the drugs were obtained by further dilution of stock solution. Working standards were procured from reputed companies with the certificate of analysis. Acetonitrile and methanol of HPLC grade were obtained from Merck Limited, Mumbai. Potassium dihydrogen phosphate was of AR grade, obtained from S. D. Fine-Chem. Ltd, Mumbai. The mobile phase was filtered through 0.45 μ membrane filter paper and degassed before use.

The apparatus used was a Jasco HPLC-900 series equipped with PU-980 intelligent pump, AS-950 intelligent auto-sampler (1-100 μl) and UV-975 intelligent UV/Vis detector with 8 μl flow cell. The analytes were separated on a μBondapak C_{18} (300x3.9 mm) 10 μm column with wavelength programming and analyzed using Borwin chromatographic software.

For the method development, to start with C_{18} column has been selected, simple mobile phase of acetonitrile and water had been tried in various proportion, they were not found ideal. As pKa values of both the drugs were found to be critical, phoshphate buffer had been used. Mobile phase of 10 mM potassium dihydrogen phosphate buffer and acetonitrile in proportion 40:60 with pH 6.0 was found to be sufficient for eluting both drugs with good resolution. Chromatograms for individual drugs were found to have all the merits of ideal chromatography, but in solution of the dosage form the peak response of metformin was found to be discontinuous due to higher concentration. The areas were specified, but there was no linearity between different concentrations of metformin when chromatographed in the mixture. As the molar extinction coefficient of metformin was significantly low at 265 nm, it was chosen for the measurement of absorbance, while for glimepiride the absorbance was measured at 230 nm. The wavelength programming was done as, for 0-5.8 min at 265 nm, 5.81-9.0 min at 230 nm and 9.01-11 min at 265 nm. A typical chromatogram of metformin and glimepiride 500 and 2.0 μg/ml obtained at the optimized conditions is shown in the fig. 1 and the chromatographic figures of merit in Table 1.

For the linearity data, concentrations ranging for 400, 425, 450, 475, 500, 525, 550, 575, 600 μg/ml solution for

TABLE 1: CHROMATGRAPHIC FIGURES OF MERIT FOR HPLC SEPARATION OF TWO DRUGS.

Drug	Metformin	Glimepiride
Retention time (t_R, min)	3.42	6.22
Relative retention time (RRT)	1	1.18
Capacity factor (k')	0.93	1.9
Selectivity factor (∝)	-	2.04
Resolution (R)	-	4.64
Tailing factor (T)	1.0	1.0
No. of plates (N)	1273	3364
Height equivalent of theoretical plate (h, cm)	0.0236	0.0089

Figures denote ideal chromatographic separation, but only the efficiency is lower.

TABLE 2: LINEAR REGRESSION LEAST SQUARE FIT DATA FOR HPLC ASSAY OF TWO DRUGS.

Drug	Metformin	Glimepiride
Linear dynamic range (μg/ml)	400-600 μg/ml	1.6-2.4 μg/ml
Slope (m)	107.36	3251.83
Intercept (b)	148.11	86.78
Correlation coefficient (r)	0.9999	0.9999
S. D. for slope (Sm)	2.28	79.39
S. D. for intercept (Sb)	1151.20	160.11
Syx	442.19	51.98

y = mx + b (where y = peak response, m = slope, x = concentration (μg/ml), b = intercept).

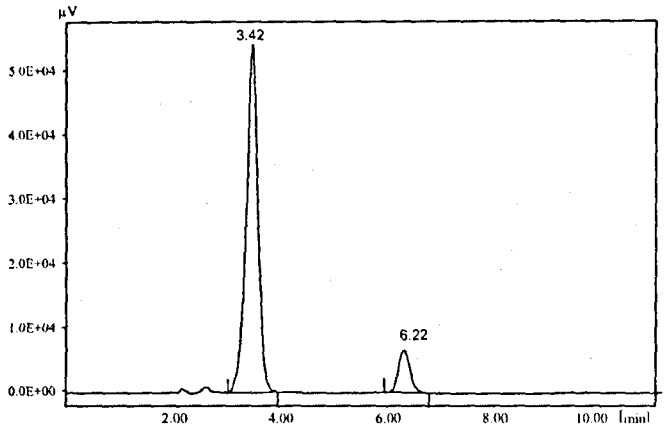

Fig. 1: Typical Chromatogram of metformin and glimepiride.

Typical Chromatogram of separation of metformin (500 μg/ml, t_R = 3.42 min) and glimepiride (2 μg/ml, t_R = 6.22 min) under optimized conditions.

metformin and 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4 μg/ml for glimepiride were prepared and injected. Response was measured as the peak height and it was found linear over the range. The data was analyzed using linear regression least squares fit data and the results are given in Table 2.

For the assay of tablets, locally available Glimtide of Meno Pharmaceuticals Pvt. Ltd., was procured and used. Twenty tablets were powdered and homogenized. A quantity of the powder equivalent to 500 mg of metformin and 2 mg of glimepiride was weighed and dissolved in 100 ml of methanol. From this solution 1.0 ml of filtrate was diluted to get a solution having concentration 500 μg/ml of metformin and 2.0 μg/ml of glimepiride. Twenty microliters of this solution was injected into the chromatograph under the conditions specified. The analyte peaks were identified by comparison with observed retention times with those of respective standards. The peak response, measured as peak heights were related to the slopes and intercepts from calibration data to calculate concentration of both the drugs. From a series of five experiments the mean values obtained for the Glimtide dosage form were, for metformin 496±1.85 and for glimepiride 1.98±0.12 mg against the labeled amount of 500 and 2 mg, respectively. Commonly used excipients such as starch, microcrystalline cellulose, talc, lactose, and hydroxy propyl methyl cellulose. do not interfere in the method.

Accuracy and precision were estimated by assaying

TABLE 3: ACCURACY AND PRECISION OF THE HPLC METHOD FOR SIMULTANEOUS ASSAY OF THE TWO DRUGS.

Drug	Original conc. (μg/ml)	Conc. added (μg/ml)	Total conc. (μg/ml)	Conc. found (μg/ml)	Error (%)	Recovery (%)	C.V. (%)
Metformin	450	50	500	479	4.16	95.8	4.23
	450	75	525	517	1.56	98.8	2.89
	450	100	550	546	0.69	99.3	1.03
Glimepiride	1.5	0.25	1.75	1.65	5.71	94.3	6.08
	1.5	0.5	2.0	1.96	2.00	98.0	4.14
	1.5	0.75	2.25	2.23	0.89	99.1	1.95

% Error=[Difference in conc. added and conc. found/conc. added]x100.

solution of the dosage form spiked with mixture containing known amount of these two drugs. Each experiment was repeated five times and mean recovery and percentage coefficient of variation (% C.V.) are given in Table 3. As can be seen from the table, recovery values are all between 99.5 to 101% and % C.V. values of replicate estimation do not exceed 3 %. A simple, sensitive and rapid method has been developed for the isocratic separation and simultaneous estimation of metformin and glimepiride in bulk and pharmaceutical dosage forms by using wavelength programming in HPLC.

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Extractive Spectrophotometric Method for the Determination of Clarithromycin

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A simple extractive spectrophotometric method has been developed for the estimation of *clarithromycin in both pure and pharmaceutical dosage forms.* The method is based on the formation of an ion-pair complex of the drug with bromocresol green, which is extracted into chloroform. The absorbance of the chloroform layer is measured at 415 nm against a reagent blank. The method has been statistically evaluated.

Clarithromycin (CMN), 6-methoxy erythromycin[1,2] is used for the treatment of streptococcal pharyngitis, respiratory tract infections and acute sinusitis. It is currently being evaluated for the treatment of some refractory infections in AIDS patients[2]. A few analytical methods based on ion-pair HPLC[3], capillary electrophoresis[4] and RP- HPLC[5,6] to estimate the drug in gastric juice, plasma, serum and urine appeared in literature. Some HPLC[7,8] and colorimetric[9-13] methods have also been reported for the assay of CMN in various dosage forms and in bulk drugs. The authors report the development of a simpler extractive spectrophotometric method based on the formation of an ion-pair complex with bromocresol green (BCG) for its determination.

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A Systronics UV/Vis spectrophotometer (model 117) with 10 mm matched quartz cells was employed for all the spectral measurements. Chemicals used in the investigation were of analytical grade. The BCG solution (0.5%) was prepared by dissolving 500 mg of the dye in 100 ml of distilled water. This solution was shaken with chloroform to remove any chloroform soluble impurities. An accurately weighed quantity of clarithromycin (100 mg) was dissolved in 100 ml of methanol to obtain a stock solution of 1 mg/ml strength. This solution was further diluted with distilled water to get a working standard solution containing 100 μg/ml of the drug.

Aliquots of the standard drug solution ranging from 0.5-3.0 ml were transferred into a series of 150 ml separating funnels. To each funnel, 2 ml of HCl (0.1 N) and 1 ml of the dye solution were added and the total volume was adjusted