tent of nalidixic acid and metronidazole were calculated using simultaneous equations. The results are shown in Table 1.

Recovery studies for tablet formulations were then done by accurately weighing the quantity of tablet powder equivalent to 100 mg of nalidixic acid and transferring to 50 ml volumetric flask and then it to 10 mg of pure nalidixic acid and 10 mg of metronidazole were added accurately. The mixture was then dissolved in methanol with slight warming and diluted to 50.0 ml. The solution was then filtered and 10 ml portion of the filtrate was diluted to 100 ml with 0.1N hydrochloric acid. A portion (5.0 ml) of the resultant solution was further diluted to 100 ml with 0.1 N hydrochloric acid. The absorbance of the solution was measured at 257 nm and 277 nm against a blank. The content of nalidixic acid and metronidazole were calculated using simultaneous equations. The percent recovery was then calculated with respect to the amount of pure nalidixic acid and metronidazole added.

For recovery studies for suspension formulation, the same procedure as detailed under recovery studies for tablet was repeated by weighing the quantity of suspension equivalent to about 50 mg of nalidixic acid and adding accurately weighed quantities of nalidixic acid and metronidazole. The results are shown in Table 1.

Accuracy of the analysis was determined by calculating recovery of nalidixic acid and metronidazole by standard addition method. The results indicated that the recovery of nalidixic acid ranged between 99.0-100 and that of metronidazole between 98.0-100, ensuring that the method is accurate and reproducible. It also appears that the excepients present in formulation do not interfere in the proposed method. In addition to this, the method is simple rapid and cost effective. Hence, the method may be employed for routine quality control of formulations containing nalidixic acid and metronidazole.

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Validation of a RP-LC Method for Simultaneous Determination of Paracetamol, Methocarbamol and Diclofenac Potassium in Tablets

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A simple, fast, precise and accurate liquid chromatographic method was developed for the simultaneous estimation of paracetamol, methocarbamol and diclofenac potassium in tablets. Drugs were chromatographed on a reverse phase Hypersil C_{18} column using a mobile phase, 25 mM

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phosphate buffer (pH adjusted to 7.0±0.1) and acetonitrile in the ratio of 65:35 v/v. The flow rate was 1.2 ml/min and the effluent was detected at 225 nm. Chlormezanone was used as an internal standard. The retention time of paracetamol, methocarbamol and diclofenac potassium were 2.74, 3.82 and 6.05 min, respectively. The method was linear (correlation co-efficient was more than 0.999), precise (percentage residual standard deviation for 0.15 for paracetamol, 0.17 for methocarbamol and 0.16 for diclofenac potassium), accurate (overall mean average recovery yields: 99.9% for paracetamol, 100% for methocarbamol and 101% for diclofenac potassium) and selective. Due to its simplicity and accuracy, the method was suitable for routine quality control analysis of these drugs in combined dosage form.

Paracetamol (PAR), chemically 4- hydroxy acetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic. Methocarbamol (MTL) is 1,2-propanediol-3-(2-methoxy phenoxy)-1-carbamate which is used as skeletal muscle relaxant. Diclofenac potassium (DCL) is used as non steroidal antiinflammatory drug and it is chemically potassium [o-(2,6-dichloroanilino) phenyl] acetate. A combination of 325 mg of paracetamol, 500 mg of methocarbamol and 50 mg of Diclofenac potassium is available as a combination tablets (Robinaxol-D, Khandelwal laboratories, Mumbai). This combination is used for spasm and pain associated with musculoskeletal disorders.

PAR is official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia and DCL is official in British Pharmacopoeia and United States Pharmacopoeia. Methods have been reported for the simultaneous estimation of PAR and MTL by UV[1], GC[2] and HPLC[1,3,4]. Various analytical methods were reported for the simultaneous estimation of PAR and DCL by reverse phase HPLC[5-7] methods. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. The present work describes a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of PAR, MTL and DCL in combined dosage forms.

A Shimadzu® HPLC (LC-10 AT VP) system with Class VP 5.02 version software was used for the analysis. The column used was Hypersil C 18 (25 cm x 4.6 mm i.d., 5 µ) and was eluted with filtered and degassed mobile phase consisting 25 mM phosphate buffer (pH 7.0) and acetonitrile in the proportion of 65:35 v/v. The flow rate of mobile phase was 1.2 ml/min and the detection was performed at 225 nm. The separation was carried at room temperature of 20±2°. The drugs were obtained from Sun Pharma, Mumbai as gift samples and the drugs were used without further purification. Sodium dihydrogen orthophosphate AR grade, phosphoric acid AR grade and acetonitrile HPLC grade all were procured from S. D. Fine Chemicals, Mumbai.

Standard stock solutions of PAR (325 µg/ml), MTL (500 µg/ml) and DCL (50 µg/ml) were prepared using a mixture of acetonitrile and water (1:1 v/v). From the standard stock solutions, mixed standard solution was prepared to contain 32.5 µg/ml PAR, 50 µg/ml MTL, 5 µg/ml DCL and 25 µg/ml of chlormezanone as an internal standard. Test solution was prepared by grinding 10 tablets and powder equivalent to 32.5 mg of PAR, 50 mg of MTL and 5 mg of DCL (one tenth of labeled claim) was weighed and added with 25 mg of chlormezanone as an internal standard. Extraction was carried out with a mixture of acetonitrile and water in a ratio of 1:1 v/v in three quantities of 20 ml and the solution was sonicated for 10 min and the volume was made up to 100 ml and filtered. From the filtrate solution, 5 ml was diluted to 50 ml by using diluent to obtain test solution.

With the optimized chromatographic conditions, a steady baseline was recorded. The mixed standard solution was injected and the chromatogram was recorded. The retention time of PAR, MTL, DCL and chlormezanone was 2.74, 3.82, 6.05 and 8.51min, respectively. This procedure was repeated for the test solution obtained from the pharmaceutical dosage form. The response factor (peak area ratio of standard to internal standard peak area) of standard and sample solution was calculated. The resultant concentrations will be approximately made as 32.5 µg/ml of PAR, 50 µg/ml of MTL and 5 µg/ml of DCL (theoretical value).

The main problem in the development of HPLC method for the analysis of PAR, MTL and DCL was to find a suitable combination of mobile phases to separate the component. The preliminary isocratic studies on a reverse phase C_{18} column with different mobile phase combination of acetonitrile and phosphate buffer pH 7.0 were studied. The wavelength

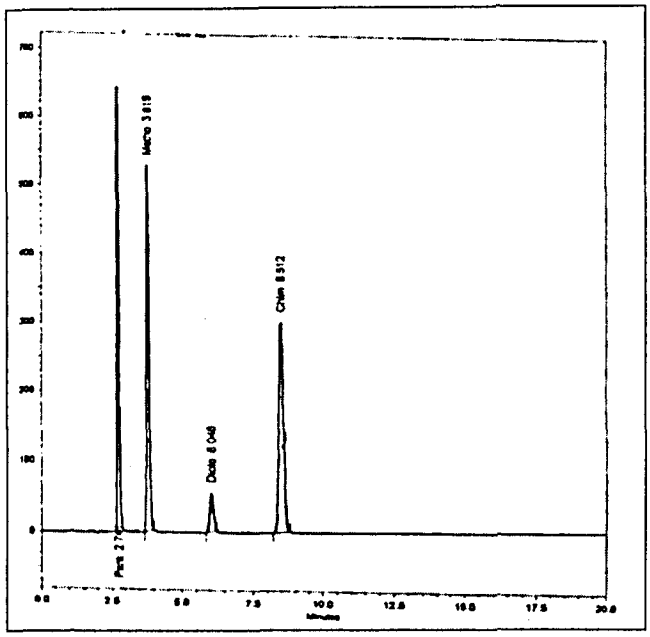

Fig. 1: Typical chromatogram of sample solution

Chromatogram showing sample solution of commercially marketed tablets along with Chlormezanone as an internal standard

selected was 225 nm in which optimal absorbance of all the drugs were good. The proposed chromatographic condition was optimized method for the evaluation of PAR, MTL and DCL in tablets. The pH changes in phosphate buffer from pH 2.0 to 7.0 did not considerably affected PAR and MTL whereas DCL has higher retention in more acidic pH. The increase in retention of DCL may be due to the ionization of DCL at neutral pH. It was found that increase in pH, decrease the retention of DCL. Triethylamine as peak modifier (0.5% v/v) does not change the peak shape considerably. The reason may be due to the absence of silanol effects of the column because of neutral or acidic nature of the selected three drugs. The mobile phase was optimized with 25 mM phosphate buffer pH 7.0 and acetonitrile in the proportion of 65:35 v/v. The typical chromatograms obtained with test solutions were shown in the fig.1. The retention time of PAR, MTL and DCL were 2.74, 3.82 and 6.05 min respectively. The capacity factor k' obtained for PAR, MTL and DCL were 0.44, 1.00 and 2.17 respectively. The resolution, capacity factor and tailing factor were shown in the table 2. The proposed method was validated as per ICH guidelines[9]. Accuracy of the method was studied by recovery studies[8]. To the pre-analysed sample of tablet (test solution) containing 32.5 µg of PAR, 50 µg of MTL, 5 µg/ml of DCL and 25 µg/ml of chlormezanone (theoretical value) as an internal standard were added with reference standard drugs at the level of 25%, 50% and 100% of the label claim. The average recovery of PAR, MTL and DCL were 99.9, 100.1 and 100.9%, respectively. Since the results obtained were within the acceptable limits ±2% range the method was found to be accurate. The use of internal standard will indicate the extraction errors and the multi component dosage forms can be quantitated effectively. Chlormezanone is selected as an internal standard because of the similar retention to the analytes, and had similar detector response to the analyte concentration used. System precision and method precision experiments showed the method was precise. The system precision was demonstrated by six repeated injections of

TABLE 1: ANALYSIS OF FORMULATIONS AND RECOVERY STUDIES

Name of the Drug	Label claim (mg/tablet)	Amount taken for assay (µg/ml)	Amount Found (µg/ml)	%Label claim	% Recovery
Paracetamol	325	32.5	32.2 (0.12)	99.9 (0.87)	99.9 (0.46)
Methocarbamol	500	50	49.6 (0.46)	99.3 (0.61)	100. (0.96)
Diclofenac potasium	50	5	4.9 (0.29)	97.9 (1.16)	100.9 (1.21)

The amounts recovered and mean percentage recovery of each levels (n=6) PAR, MTL and DCL were given. The percentage relative standard deviations were given in the bracket. PAR stands for Paracetamol, MTL stands for Methocarbamol and DCL stands for Diclofenac Potassium.

TABLE 2: SYSTEM SUITABILITY STUDIES

Parameter	Paracetamol	Methocarbamol	Diclofenac Potassium	Chlormezanone
Theoretical Plates/meter	27483	31662	38031	48878
Resolution	-	7.09	10.62	8.86
Asymmetry (10%)	1.05	1.09	1.03	1.05
Capacity factor	0.45	1.06	2.17	3.46
LOD (ng/ml)	10	10	25	5
LOQ (ng/ml)	25	25	50	10

The system suitability parameters of Paracetamol, Methocarbamol and Diclofenac potassium were given.

standard solution and the response factor (peak area of the drug peak to the peak area of internal standard) was calculated. The percentage residual standard deviation of PAR, MTL and DCL were 0.15, 0.17 and 0.16 respectively. The low percentage residual standard deviation of the analytes showed that the method was precise. The method was found to be linear in the range of 70 to 130% of assay concentration of all drugs and the correlation co-efficient (r^2) was found to be >0.999. The calibration curve was plotted using response factor (peak area of the drug to the peak area of internal standard) Vs concentration of the standards solutions. The system suitability studies were also carried out to determine column efficiency (Theoretical plates per meter), limit of detection (LOD), limit of quantitation (LOQ) were given in the table 2. The peaks of PAR, MTL and DCL were symmetrical and the symmetry factor for the analytes peaks was lesser than 2%. The selectivity was demonstrated showing that peaks of analytes were free of interference from excipients indicating that the proposed method was selective.

The developed HPLC method provides a convenient and efficient method for the separation and determination of PAR, MTL and DCL in combined dosage forms. No interference was found from excipients used in the tablet formulation and the method is simple and has run time of 10 min, which make it especially suitable for routine quality control analysis. The reverse phase HPLC method was linear, precise, accurate and selective and can be employed for the assay of PAR, MTL and DCL in dosage forms.

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