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Estimation of Acetaminophen, Dextropropoxyphene and Oxyphenbutazone in Combined Dosage Forms by HPLC Method

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A simple and precise reversed phase HPLC method was developed for the simultaneous estimation of acetaminophen, dextropropoxyphene and oxyphenbutazone in tablet formulations. The method was carried out on a Kromasil® C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile:0.5% triethylamine (adjusted to pH 3.5 using orthophosphoric acid), (45:55 v/v) at a flow rate of 1.2 ml/min. Detection was carried out at 220 nm. Chlormezanone was used as internal standard. The retention time of acetaminophen, dextropropoxyphene, chlormezanone and oxyphenbutazone was 2.26, 3.72, 4.96 and 10.67 min, respectively. The validation of the proposed method was also carried out. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Acetaminophen, chemically 4-hydroxy acetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic. Dextropropoxyphene, chemically (+)-(1S,2R)-1-benzyl-3-dimethylamino-2-methyl-1-phenylpropyl propionate, is a centrally acting opioid analgesic. Oxyphenbutazone, chemically (RS(-4-butyl-1-(4-hydroxyphenyl)-2- phenylpyrazolidine-3,5-dione monohydrate is an analgesic, antipyretic and antiinflammatory agent. When these three are used together, the action of acetaminophen is set in earlier and provides pain relief before the effects of dextropropoxyphene and oxyphenbutazone are felt. A combination of 250 mg of acetaminophen, 32.5 mg of dextropropoxyphene and 100 mg of oxyphenbutazone is available commercially as tablets. This combination may be used for myalgia, aches and pains, severe rheumatoid arthritis, neuralgia and back pains.

Many method[1-7] have been described in the literature for the determination of acetaminophen, dextropropoxyphene and oxyphenbutazone, individually. 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 reversed phase HPLC method for the simultaneous estimation of acetaminophen, dextropropoxyphene and oxyphenbutazone in combined dosage forms.

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EXPERIMENTAL

Triethylamine AR grade, orthophosphoric acid AR grade and acetonitrile of HPLC grade were supplied by S.D. Fine Chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standards of acetaminophen, dextropropoxyphene and oxyphenbutazone were obtained from Wockhardt Ltd, Mumbai.

Chromatographic conditions:

A Shimadzu® HPLC (LC-10AT *VP*) system was used for the analysis. The method was carried out on a Kromasil® C_{18} (25 cm x 4.6 mm i.d., 5 µ) column as a stationary phase and acetonitrile:0.5% triethylamine (pH adjusted to 3.5 using orthophosphoric acid) (45:55 v/v) as the mobile phase at a flow rate of 1.2 ml/min. A

Rheodyne 7725 injector with a 20 µl loop was used for the injection of samples. Detection was done at 220 nm with a sensitivity of 0.20 AUFS. The mobile phase was filtered through a 0.45 µ membrane filter and degassed. The separation was carried out at the room temperature of about 20°.

Preparation of solutions:

Standard stock solution of 1 mg/ml of acetaminophen, dextropropoxyphene, chlormezanone and oxyphenbutazone were prepared separately using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solutions, mixed standard solution was prepared to contain 25 µg/ml of acetaminophen, 3.25 µg/ml of dextropropoxyphene and 10 µg/ml of oxyphenbutazone containing 20 µg/ml of chlormezanone as internal standard.

Twenty tablets, each containing 250 mg of acetaminophen, 32.5 mg of dextropropoxyphene and 100 mg of oxyphenbutazone were weighed and finely powdered. A quantity of powder equivalent to 25 mg of acetaminophen, 3.25 mg of dextropropoxyphene and 10 mg of oxyphenbutazone was weighed and transferred to a sintered glass crucible. To this 20 ml of 1 mg/ml of chlormezanone was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 25 µg/ml of acetaminophen, 3.25 µg/ml of dextropropoxyphene and 10 µg/ml of oxyphenbutazone (theoretical value) containing 20 µg/ml of chlormezanone as internal standard and this solution was used for the estimation.

Assay method:

With the optimised chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of acetaminophen, dextropropoxyphene, chlormezanone and oxyphenbutazone was found to be 2.26, 3.72 4.96 and 10.67 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and the internal standard peak area) of the standard solution and the sample solution were calculated. The concentration of the drugs were calculated (Table-1) using the following formula.

$$\text{Conc. of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the std}} \times \text{Concentration of standard}$$

Method Validation:

Accuracy of the method was studied by recovery experiments. To the powdered tablet formulation (25 mg of acetaminophen, 3.25 mg of dextropropoxyphene and 10 mg of oxyphenbutazone), the 20 ml of 1 mg/ml of chlormezanone solution and reference standard drugs were added at the level of 25%, 50% and 100% of the label claim. The extraction of drugs was followed using sample preparation procedure and these were analysed. The percentage recovery was calculated and presented in Table-1. Precision of the method was demonstrated by repeatability studies. This was done by injecting consecutively the standard solution for 10 times and passing them through the assay procedure.

Linearity and range of the method was determined

TABLE 1 : ANALYSIS OF FORMULATIONS AND RECOVERY STUDIES

Drug	Amount (mg/tablet)		% Label	%
	Labelled	Found*	Claim*	Recovery*
Acetaminophen	250	250.6 ±2.6784	100.24 ±1.071	100.13 ±0.1324
Dextropropoxyphene	32.5	33.0 ±0.8940	101.53 ±2.34	99.95 ±0.1024
Oxyphenbutazone	100	97.24 ±1.1764	97.24 ±1.1764	99.98 ±0.2014

*Mean ±SD of 6 observations

by analysing mixed standard solutions containing 12.5 to 37.5 µg/ml of acetaminophen, 1.63 to 4.88 µg/ml of dextropropoxyphene and 5 to 15 µg/ml oxyphenbutazone (50 to 150% of targeted level of the assay concentration) containing 20 µg/ml of chlormezanone as internal standard, respectively. The calibration curve was plotted using response factor Vs concentration of the standard solutions, the values are presented in Table 2. The limit of detection (LOD) and limit of quantification (LOQ) of the method was determined by injecting progressively low concentrations of the standards solutions with the optimised chromatographic conditions.

RESULTS AND DISCUSSION

The chromatograms of mixed standard solution and sample solution are presented in Fig. 1. The accuracy of the method was determined by recovery studies. The recovery studies were carried out and the percentage recovery was calculated. From the data obtained, recoveries for the standard drugs were considered accurate.

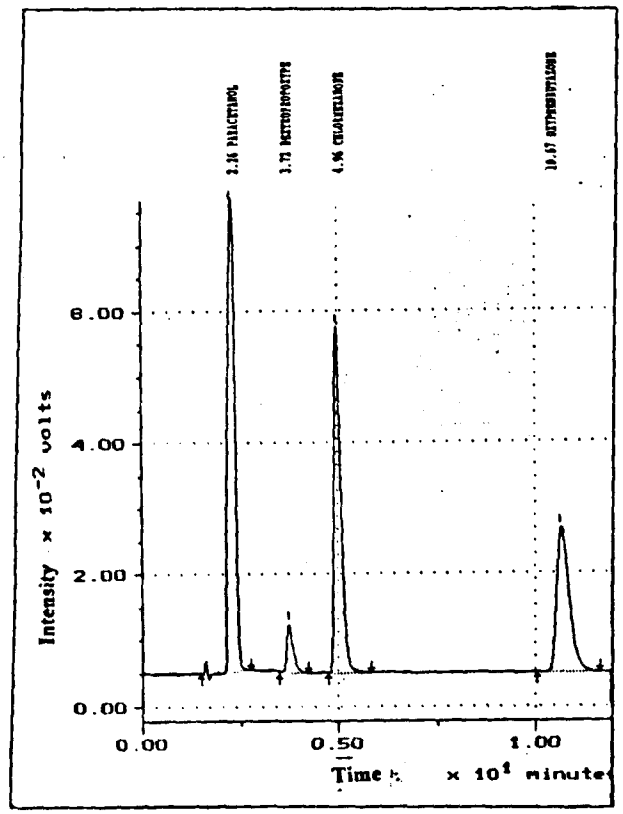

Fig. 1 : Chromatogram of Acetaminophen, dextropropoxyphene and oxyphenbutazone

TABLE 2 : LINEARITY AND RANGE

Internal standard Peak area (Chlormezanone 20 µg/ml)	Acetaminophen Concentration (µg/ml)	Acetaminophen Peak area	Acetaminophen Response factor	Dextropropoxyphene Concentration (µg/ml)	Dextropropoxyphene Peak area	Dextropropoxyphene Response factor	Oxyphenbutazone Concentration (µg/ml)	Oxyphenbutazone Peak area	Oxyphenbutazone Response factor
632503	12.50	418286	8.2664	1.6250	41472	0.1065	5.0	240281	1.8990
	18.75	627428	18.5995	2.4375	62205	0.2397	7.5	360424	4.2737
	25.00	836572	33.0659	3.2500	82940	0.4261	10.0	480565	7.5978
	31.25	1045715	51.6655	4.0625	103675	0.6658	12.5	600708	11.8716
	37.50	1254858	74.3983	4.8750	124415	0.9589	15.0	720847	17.0951

TABLE 3 : SYSTEM SUITABILITY STUDIES

S.No.	Parameter	Acetaminophen	Dextropropoxyphene	Chlormezanone	oxyphenbutazone
1	Theoretical plates*	226	2443	6033	17789
2	Resolution	-	4.13	3.59	9.82
3	Peak asymmetry	1.0	1.02	1.02	1.03
4	LOD (ng/ml)	10	25	25	50
5	LOQ (ng/ml)	50	100	100	250

* per column length used

The precision data shows that the reproducibility of the assay procedure was satisfactory. The concentration range from 12.5 to 37.5 µg/ml for acetaminophen, 1.63 to 4.88 µg/ml for dextropropoxyphene and 5.0 to 15.0 µg/ml for oxyphenbutazone were examined by the assay procedure and the calibration curves were plotted. The calibration curve shows linear response over the range of concentration used in the assay procedure. The calibration curve passes through the origin, which justifies the use of single point calibration and the proximity of all points to the calibration line demonstrated that the method has adequate linearity to the concentration of the analyte.

The limit of detection (LOD) for acetaminophen, dextropropoxyphene, chlormezanone and oxyphenbutazone was found to be 10 ng/ml, 25 ng/ml, 25 ng/ml and 50 ng/ml, respectively and the limit of quantification (LOQ) was 50 ng/ml, 100 ng/ml, 100 ng/ml and 250 ng/ml for acetaminophen, dextropropoxyphene, chlormezanone and oxyphenbutazone (Table-3). The ruggedness of the method was determined by carrying out the experiment on different instruments like Spectra Physics HPLC, Shimadzu HPLC (LC-10AT *VP*) and Water's HPLC by different operators using different columns of similar type like µBondapak, Hypersil and Nucleosil. Robustness of the method was determined by making slight changes in the chromatographic conditions. Further there is no interference due to excipients. The system suitability studies were also carried out to determine column efficiency, resolution and peak asymmetry (Table-3). The proposed HPLC methods is simple, accurate, precise, linear, rugged and rapid. Hence this method can be applied for the quality control of raw materials, formulations and in dissolution studies.

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