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Simultaneous estimation of naproxen and dexamethasone sodium phosphate in pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A modified simple, selective, rapid, precise reversed phase high performance liquid chromatography method has been developed and validated for the simultaneous estimation of naproxen and Dexamethasone Sodium. The separation was made in a HYPERSIL C_{18}, 250 X 4.6 mm, 5μ column using Mixed Phosphate buffer (1.36 gms of potassium Dihydrogen phosphate and 0.6gms of DiPotasssium hydrogen phosphate in 1 Liter water pH 6.8±0.1): Methanol in the ratio 45: 55 at 273 nm. The mobile-phase flow rate and the sample volume injected were 1 ml/min and 20 μl, respectively. Retention time of naproxen and Dexamethasone Sodium was found to be 3.383 and 4.896 minutes respectively. The standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (naproxen r=0.999 & Dexamethasone r=0.998) was observed between the concentration range of 20-80 µg/mL. The assay of naproxen was found to be 99.84% & the assay of Dexamethasone was found to be 99.85%.From the recovery studies it was found that about 101% of drug was recovered which indicates high accuracy of the method. It is suitable for the routine analysis of naproxen and Dexamethasone in pharmaceutical dosage form. The limit of detection (LOD) and limit of quantification (LOQ) for naproxen was found to be 3.7367µg/mL & 11.3236µg/mL. The limit of detection (LOD) and the limit of quantification (LOQ) for Dexamethasone were found to be 0.5502µg/mL &1.66736µg/mL. It was concluded that in the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved.

Keywords: Naproxen, Dexamethasone, HPLC, Limit of quantification (LOQ), Limit of detection (LOD)

INTRODUCTION

Naproxen (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid is a highly potent member of nonsteroidal anti-inflammatory drugs. The compound shows potent prostaglandin cyclooxygenase inhibitory activity [1]. Naproxen, when administered intramuscularly or orally, is a safe and effective analgesic agent for the short-term management of acute postoperative pain and can be used as an alternative to opioid therapy [2]. Dexamethasone sodium phosphate (DSP) is a highly selective glucocorticoid which is widely used in ocular inflammatory diseases. Its chemical name is 9- fluoro-11b, 17, 21-trihydroxy-16α-methylpregna-1, 4- diene-3, 20- dione 21-(dihydrogen phosphate) disodium salt. [3]. Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several

advantages like rapidity, specificity, accuracy, precision and ease of automation in this method.

High-performance liquid chromatography [HPLC] is now one of the most important tools in analytical chemistry. It has the ability to separate, identify, quantitated the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in few concentrations as low as parts per trillion may easily be identified. HPLC can be, has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, industrial chemicals. [4,5,6].

MATERIALS AND METHODOLOGY

Materials

HPLC **:** Waters HPLC 2 2695 series consisting pump, Auto sampler, UV- Visible detector, Thermostat column compartment connected with waters (alliance) Empower2 software

Balance : Sartorious Cpa225d Semi Micro Balance

Sonicator : Bio-Technics India, Mumbai

Reagents : Merck Mumbai.

Methodology

Chromatograhic conditions

Column : HYPERSIL C_{18}, 250 X 4.6 mm, 5μ,

Flow rate : 1.0mL /min

Wavelength : 273nm

Column temperature: 30°C

Injection volume: 20 μL

Run time : 10 minutes

Diluent : Mobile phase

Elution : Isocratic

Needle wash : Water: Acetonitrile 90:10 (v/v)

Mobile Phase Preparation

Mixed Phosphate buffer (1.36gms of potassium Dihydrogen phosphate and 0.6gms of Dipotasssium hydrogen phosphate in 1 Liter water pH 6.8±0.1): Methanol in the ratio 45: 55

Weigh and transfer 30 mg of Naproxen & 4 mg of Dexamethasone working standard into 100mL volumetric flask, add 20mL of diluent and sonicated to dissolve and dilute to volume with diluent.

Standard Preparation

Transfer 10ml of standard stock solution into 100ml volumetric flask and dilute to volume with diluent.

Sample Preparation

Crush to powder 20 tablets, weigh and transfer the tablet powder equal to 30 mg of Naproxen & 4 mg of Dexamethasone into 100mL volumetric flask add 20ml of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filtrate the solution through 0.45μ filter. Dilute 10ml to 100ml with mobile phase.

Procedure

Inject 20μL of blank solution, placebo solution, three times of Standard solution, Disregard peaks due to blank and placebo.

Validation

Recovery

The absolute recovery of analytical method was measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard which has not been subjected to sample pre treatment and indicates whether the method provides a response for the entire amount of analyte that was present in the sample.

$$\text{Absolute recovery} = \frac{\text{Response of an analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

Selectivity (Specificity)

The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method was determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest

concentrations, which can determined with acceptable accuracy. It was suggested that, this be set at 15% for both the upper and lower limit of quantitation respectively. Any sample concentration that falls outside the calibration range cannot be interpolated from the calibration line and extrapolation of the calibration curve was discouraged. If the concentration was over range, the sample should be diluted in drug-free matrix and re-assayed. Ability to analyze the analyte in presence of components which are expected to be present

Interferences with analyte:

- Blank interference
- Placebo interference
- Impurity interference
- Forced degradation

Acceptance Criteria

There should be no interference at the retention time of the analyte peak.

Linearity

It was the ability of the method to elicit test result that is directly proportional to analyte concentration within a given range. It is generally reported as variance of slope of regression line.

- To fix the concentration of the standard and sample solutions

- Prepare the linearity solutions in 50 -150% of target concentration
- Plot the graph of area vs. concentration and calculate the correlation coefficient.
  Acceptance criteria: The correlation coefficient should be NLT 0.999

Range

It is the interval between the upper and lower levels of analyte, which is studied. The range is normally expressed in the same units as the test results obtained by the analytical method. The ICH guidelines specify of five concentration levels.

Precision

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term 'set' is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{n-1}\sum_{i-1}^{n}\left(x_i - \overline{x}\right)^2}$$

S = Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the

standard deviation expressed as a fraction of the mean, i.e., S/X. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

$$\% \text{ Relative standard deviation} = \frac{S}{X} \times 100$$

S = standard deviation
X = mean & It is determined at three levels.

Repeatability

Precision of the method when retested by the same analysts, same test method and under same set of laboratory conditions (reagents, equipments),

within a short interval of time, the only difference being the sample.

Reproducibility

The method was carried by different analysis in different laboratories using different equipments, reagents and laboratory settings and on different days of variability of analytical results as function of analyst, day to day, laboratory to laboratory, equipment to equipment etc.

Intermediate Precision

It is determined by comparing the results of a method within the same laboratory but different days, analysts, equipment's and reagents.

Acceptance Limits

Assay results should be between 97 to 103%, %RSD should be NMT 2%.

Accuracy

Accuracy normally refers to the difference between the mean X, of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method. Accuracy was best reported as percentage bias, which is calculated from the expression

$$\%Bias = \frac{(measured\ value\ -\ true\ value)}{true\ value} \times 100$$

The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.
Acceptance Limit: 97-103%.

Limit of Detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b -is slope of the corresponding calibration curve.

$$LOD = 3\ Sa/\ b$$

Limit of Quantitation (LOQ)

The LOQ is the concentration that can be quantitativ reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = 10\ Sa/\ b$$

Where,
Sa- the estimate is the standard deviation of the peak area ratio of analyte to IS (5 injections) of the drugs. b -is slope of the corresponding calibration curve.

Ruggedness

Method Ruggedness was defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Parameters include:
- System to system variability
- Column to column variability
- Analyst to analyst variability
- Bench top stability of both standards and samples

- Refrigerator stability of both standards and samples
- Bench top stability of mobile phase

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, %organic solvent strength and buffer concentration etc. to determine the robustness of the method experimental conditions were purposely altered and chromatographic characters were evaluated.

Parameters include

- Filter validation: 2 types of filers for both standard and samples.
- Mobile phase composition variation: 90-110% variations in the organic phase
- Mobile phase ph variation: ±0.2 of method ph
- Flow rate variation: ±0.2ml of method flow rate
- Column temperature: ± 5° c of method's column temperature

System Suitability

System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. (Or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

Table 1: Method validation requirements and acceptance criteria

PRECISION	
Assay repeatability	≤ 1% RSD
Intermediate precision (Ruggedness)	≤ 2% RSD
ACCURACY	
Mean recovery per concentration	100.0% ±2.0%
LIMIT OF DETECTION	
Signal to-to-noise ratio	≥ 3:1
LIMIT OF QUANTIFICATION	
Signal to-to-noise ratio	≥ 10:1
LINEARITY/RANGE	
Correlation coefficient	>0.99±10
y-Intercept Visual	Linear
ROBUSTNESS	
System suitability met Solution stability	yes± 2% change from time zero

RESULTS AND DISCUSSION
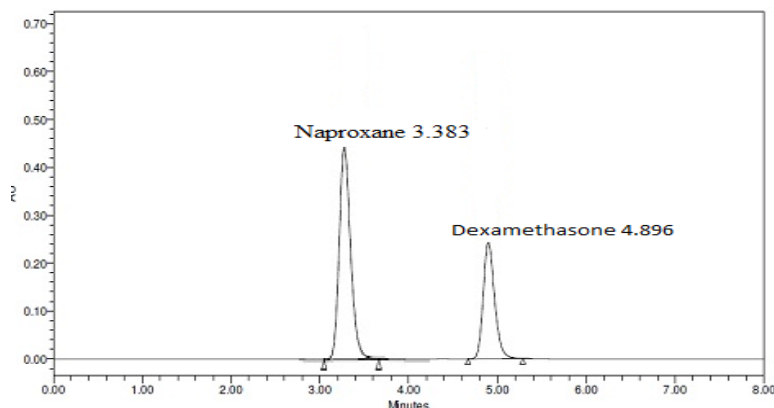
Figure 1: Naproxen Optimized Chromatogram

Precision

The relative standard deviation (%RSD) of the six assay preparations of Naproxen and Dexamethasone was calculated and it was found to be 0.06%

Table 2: Precision

Naproxen			Dexamethasone		
S. No.	RT	Area	S. No.	RT	Area
1	3.381	5352413	1	4.898	2152416
2	3.382	5362481	2	4.895	2136985
3	3.385	5328542	3	4.89	2155486
4	3.38	5410214	4	4.891	2159754
5	3.381	5381240	5	4.894	2152356
6	3.383	5341262	6	4.895	2132254
Avg	3.382	5362692	Avg	4.894	2148208.5
St.dev	0.001788854	29439.89997	St.dev	0.002927	10969.7621
%RSD	0.05	0.55	%RSD	0.06	0.51

Accuracy

To study the accuracy of the method, recovery studies were carried out. To the formulation equivalent to 10 mg of Naproxen and Dexamethasone sodium phosphate at the levels of 80%, 100% and 120% was added to pure Naproxen and Dexamethasone sodium phosphate and made up to the mark with Mobile phase and filtered through Whatmann filter paper and chromatograms were recorded. The concentration of drug present in resulting solution was determined using developed procedure and percentage recovery and percentage RSD were calculated.

Calculations

$$\frac{A_t \times W_s \times Avg.Wt \times P}{A_s \times W_t \times Claim\ Wt} = \%$$

Where,

A_t = Average area due to Naproxen Formulation peak in sample preparation

A_s = Average area due to Naproxen peak in the Standard preparation

W_s = Weight of the working standard (Naproxen)

W_t = Weight of the sample (Naproxen formulation)

P = Potency of the working standard

Avg. Wt = Average Weight

Table 3: Accuracy Values

	NAPROXEN	DEXAMETHASONE
25%spike	5421325	2135485
	5488542	2152462
	5442513	2150214
Average Placebo	5450793.333	2146053.667
%Recovery	99.42%	99.38%
50%spike	2595683	1052354
	2668935	1084521
	2865488	1068759
Average	2710035.333	1068544.667
Amount recovered	49.72	49.79
%Recovery	99.44	99.58%
100%spike	5413658	2144865
	5420156	2150891
	5422654	2124587

Average	5418822.667	2140114.333
Amount recovered	99.41	99.72
%Recovery	99.41	99.72%
150%spike	8112546	3206887
	8145784	3190145
Average	8126548	3214216
Amount recovered	8128292.667	3203749.333
%recovered	99.41	99.52%

Assay

Table 4: Assay Values of Naproxen

Standard	5335489
	5312545
	5345682
Average weight	5331239
Sample	5371254
	5342168
Average	5356711
LC	30mg
Standard weight	29.9
Sample weight	1
Standard factor	0.001
Sample factor	0.001
Standard purity	99.7
Average weight	1
Amount/ml	29.953mg
%Assay	99.84%

Table 5: Assay Values of Dexamethasone

Standard	2154698
	2148659
	2150215
Average	2151191
Sample	2155645
	2148962
Average	2152304
LC	4mg
Standard weight	4
Sample weight	1
Standard factor	0.001
Sample factor	0.001
Standard purity	99.8
Average weight	1
Amount/ml	3.994mg
%Assay	99.85%

## Linearity

Aliquots of standard Naproxen and Dexamethasone stock solution (0.2ml to 0.8ml) (1ml =100µg/mL) were taken in different 10ml volumetric flasks and diluted up to the mark with the diluents such that the final concentrations of Naproxen and Dexamethasone are in the range of 25-150µg/mL. Each of these drug solutions (20µL) was injected three times in to the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 274 nm and a calibration curve graph was obtained by plotting peak area versus concentration of Naproxen and Dexamethasone.

Table 5: Standard Calibration Values For Naproxen

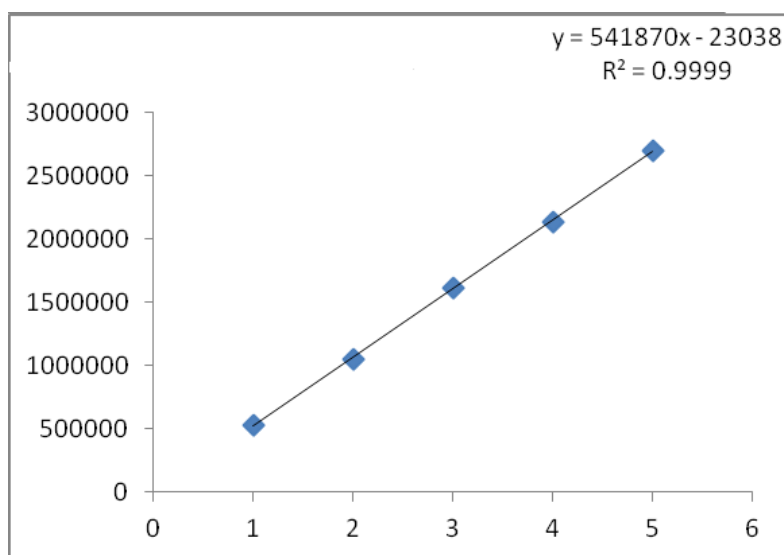| CONCENTRATION	AREA
0.0075	1322259
0.0150	2636571
0.0225	3988384
0.0300	5343309
0.0375	6686273
0.0450	8062896
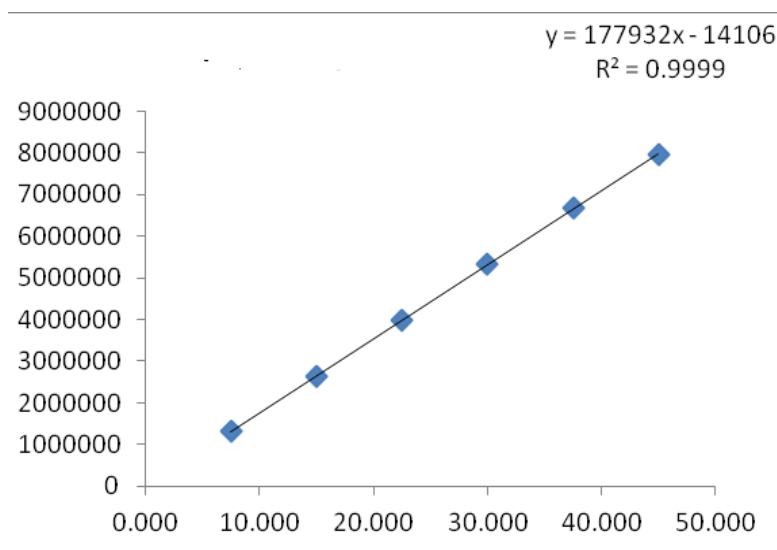
Figure 2: Standard Calibration Curve of Naproxen

Table 6: Standard Calibration Value of Dexamethasone Sp

CONCENTRATION	AREA
0.001	527153
0.002	1051037
0.003	1606349
0.004	2132599
0.005	2695722

Figure 3: Standard Calibration Curve of Dexamethasone Sp

Robustness

In order to prove that the method is robust, flow rate of the mobile phase (±0.2 ml/min) and the column temperature (±5°c) are varied. The results showed that they have passed the system suitability parameters.

Table 7: Robustness of Naproxen:

PARAMETER	RETENTION TIME (MIN)	AREA
FLOW-1	3.400	4775563
FLOW-2	3.413	4794953
TEMPERATURE-1	2.973	4715852
TEMPERATURE-2	3.929	6177487

Table 8: Robustness of Dexamethasone:

PARAMETER	RETENTION TIME (MIN)	AREA
FLOW-1	4.892	1915209
FLOW-2	4.873	1923461
TEMPERATURE-1	4.308	1917103
TEMPERATURE-2	5.692	2481425

Table 9: Performance and Detection Characteristics for the Proposed HPLC Method

PARAMETERS	OBSERVATION
%Accuracy (% Recovery)	
Naproxen	99.42
Dexamethasone SP	99.55
Precision (%RSD)	
Naproxen	0.06
Dexamethasone SP	0.06

Linearity (μg/mL)	25-150
Correlation coefficient	0.999
Theoretical plates*	
Naproxen	2398
Dexamethasone SP	3273
Height equivalent to theoretical plates	2.6×10^{-5}
LOD (μg/mL) for Naproxen & Dexamethasone SP	3.736 & 0.550
LOQ (μg/mL) for Naproxen & Dexamethasone SP	11.323 & 1.667
Assay Value (%) for Naproxen & DSP	99.84 & 99.85

CONCLUSION

The proposed method is simple, rapid, accurate, precise and specific. from the typical chromatogram of Naproxen as shown (standard & sample), it was found that the retention time for Naproxen was 3.383 min and the retention time for Dexamethasone was 4.896 min. A mixture of methanol and phosphate buffer mixture (PH 6.8) 55:45 v/v was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (Naproxen r=0.999 & Dexamethasone r=0.998) was observed between the concentration range of 20-80 μg/mL. The assay of Naproxen was found to be 99.84% & the assay of Dexamethasone was found to be 99.85%.From the recovery studies it was found that about 101% of drug was recovered which indicates high accuracy of the method. It is suitable for the routine analysis of Naproxen and Dexamethasone in pharmaceutical dosage form. The limit of detection (LOD) and limit of quantification (LOQ) for Naproxen was found to be 3.7367μg/mL & 11.3236μg/mL. The limit of detection (LOD) and the limit of quantification (LOQ) for Dexamethasone were found to be 0.5502μg/mL &1.66736μg/mL

The above proposed method obviates the need for any preliminary treatment and is simple, sensitive, and reliable and can be used for the routine determination of Naproxen and Dexamethasone formulations.

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