CHAPTER - VI

ANALYTICAL METHODS

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Method for the Estimation of Celecoxib

A spectrophotometric method based on the measurement of absorbance at 254 nm in phosphate buffer of pH 7.4 was used in the present study for the estimation of celecoxib.

Standard Solution

100 mg of celecoxib was dissolved in methanol in a 100 ml of volumetric flask and the solution was made up to volume with methanol

Procedure

The standard solution of celecoxib was subsequently diluted with phosphate buffer of pH 7.4 to obtain a series of dilutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 μg of celecoxib in 1 ml solution. The absorbance of these solutions was measured in Shimadzu – UV Pharmaspec1700, UV-Vis Spectrophotometer at 254 nm using phosphate buffer of pH 7.4 as blank. The concentration of celecoxib and the corresponding absorbances are given in Table 6.1. The absorbances were plotted against concentration of celecoxib as shown in Fig. 6.1.

Validation of the Method

1. Reproducibility

Reproducibility of the above method was studied by analyzing six individually weighed samples of celecoxib. The percent relative standard deviation (RSD) of the determinations found to be less than 1.0%.

2. Interference Study

The interference in the above method by the excipients used in the present investigation was studied by testing their effects individually. Accurately weighed amounts of celecoxib and excipients in 1 : 1 ratio were mixed thoroughly. From each an accurately weighed mixture equivalent to 50 mg of celecoxib was assayed by the method described above. The celecoxib contents were calculated using the calibration curve (Fig.6.1) and the results are given in Table 6.2.

Discussion

The method obeyed Beer's law in the concentration range of 0 - 20 µg/ml. Low RSD (< 0.25) values ensured reproducibility of the method. The results given in Table 6.2 indicated that none of the excipients used in the study interfere in the method of estimation. Thus the method was found to be suitable

for the estimation of celecoxib contents in various products and *in vitro* dissolution studies.

Table 6.1

Calibration Curve for the Estimation of Celecoxib

Celecoxib Concentration (µg/ml)	Absorbance	
	$\bar{x}$	RSD (%)
2	0.098	0.25
4	0.217	0.22
6	0.322	0.23
8	0.427	0.25
10	0.546	0.22
12	0.630	0.19
14	0.742	0.08
16	0.833	0.15
18	0.945	0.18
20	1.043	0.19

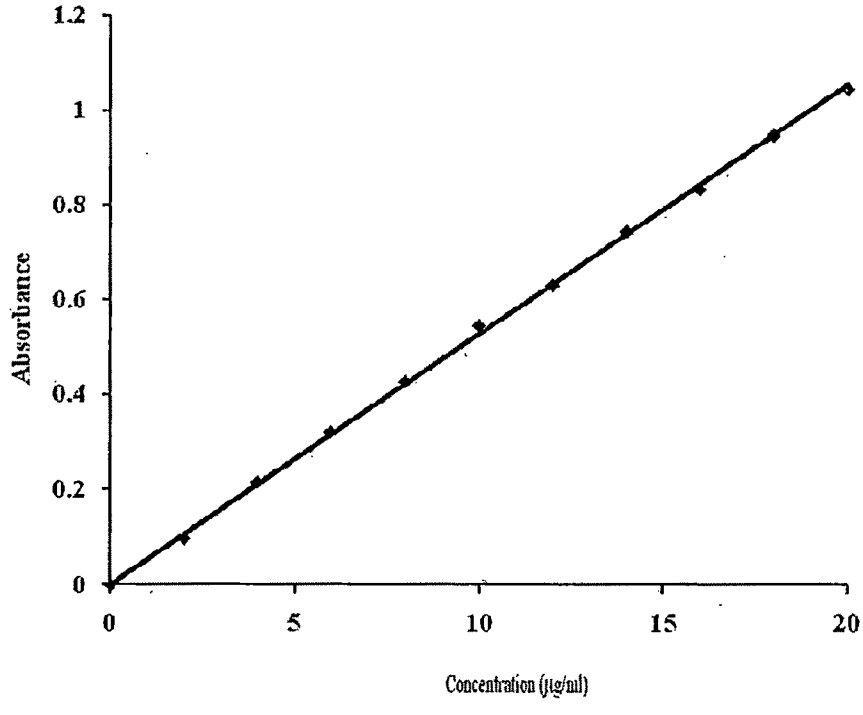

Fig 6.1

Calibration Curve for the Estimation of Celecoxib

Table 6.2

Amounts of Celecoxib Estimated in Interference Studies

Material	Amount of Celecoxib Added (mg)	Amount Estimated (mg)	Percent Estimated (Recovery)
βCD	50	49.8	99.60
HPβCD	50	49.9	99.80
HPMC	50	49.9	99.80
Carbopol	50	49.7	99.40

Method for the Estimation of Aceclofenac

A spectrophotometric method based on the measurement of absorbance at 275 nm in phosphate buffer of pH 6.8 was used in the present study for the estimation of aceclofenac.

Standard Solution

100 mg of aceclofenac was dissolved in methanol in a 100 ml of volumetric flask and the solution was made up to volume with methanol.

Procedure

The standard solution of aceclofenac was subsequently diluted with phosphate buffer of pH 6.8 to obtain a series of dilutions containing 5, 10, 15, 20 and 25 μg of aceclofenac in 1 ml solution. The absorbance of these solutions was measured in Shimadzu – UV Pharmaspec1700, UV-Vis Spectrophotometer at 275 nm using phosphate buffer of pH 6.8 as blank. The concentration of aceclofenac and the corresponding absorbances are given in Table 6.3. The absorbances were plotted against concentration of aceclofenac as shown in Fig.6.2.

Validation of the Method

1. Reproducibility

Reproducibility of the above method was studied by analyzing six individually weighed samples of aceclofenac. The percent relative standard deviation (RSD) of the determinations found to be less than 1.0%.

2. Interference Study

The interference in the above method by the excipients used in the present investigation was studied by testing their effects individually. Accurately weighed amounts of aceclofenac and excipients in 1 : 1 ratio were mixed thoroughly. From each mixture, accurately weighed powder equivalent to 100 mg of aceclofenac was assayed by the method described above. The aceclofenac content were calculated using the calibration curve (Fig.6.2) and the results are given in Table 6.4.

Discussion

The method obeyed Beer's law in the concentration range of 0 - 25 μg/ml. Low RSD (< 0.25) values ensured reproducibility of the method. The results given in Table 6.4 indicated that none of the excipients used in the study interfere the method of estimation. Thus the method was found to be suitable

for the estimation of aceclofenac contents in various products and *in vitro* dissolution studies.

Table 6.3

Calibration Curve for the Estimation of Aceclofenac

Aceclofenac Concentration (µg/ml)	Absorbance	
	$\bar{x}$	RSD (%)
5	0.15	0.25
10	0.288	0.16
15	0.425	0.08
20	0.567	0.15
25	0.703	0.21

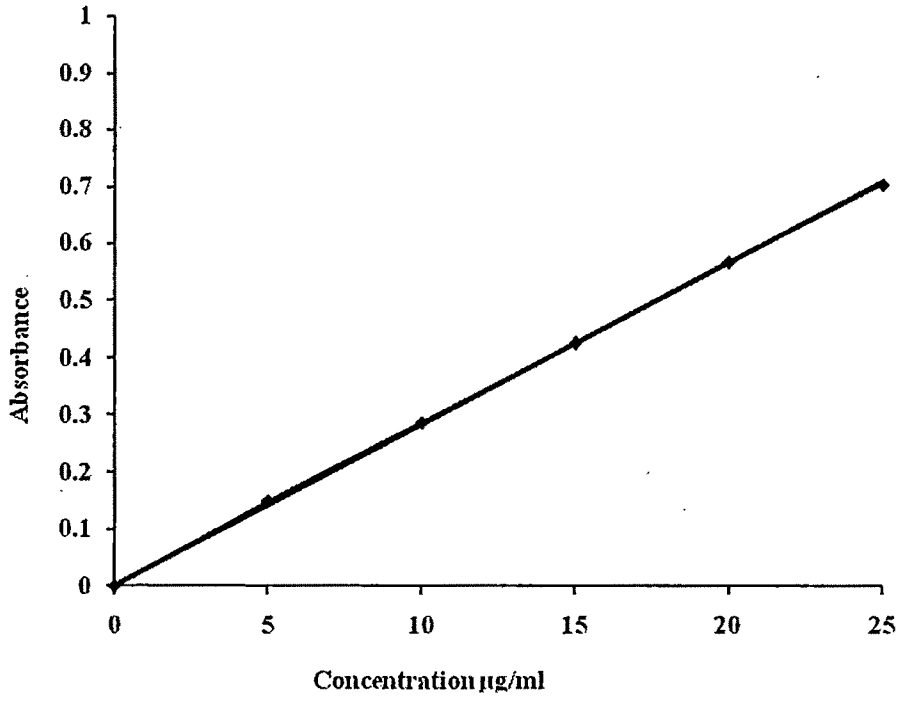

Fig. 6.2 Calibration Curve for the Estimation of Aceclofenac

Table 6.4

Amounts of Aceclofenac Estimated in Interference Studies

Material	Amount of Celecoxib Added (mg)	Amount Estimated (mg)	Percent Estimated (Recovery)
βCD	100	99.91	99.91
HPβCD	100	99.95	99.95
HPMC	100	99.99	99.99
Carbopol	100	99.98	99.98

ESTIMATION OF CELECOXIB IN PLASMA BY HPLC METHOD

A reversed phase HPLC method was used for the estimation of celecoxib concentration in plasma.

Instrumentation: The following instrumentation system was used

The HPLC system (make: M/s Shimadzu Corporation, Japan.) consisted of UV – Visible detector (Shimadzu, model: SPD – 10 AVP), C – 18 column (Phenomenex, DESC: Gemini 5µm C18 110A, Size: 250 x 4.6 mm, S/No: 288063 – 23), 2 pumps (Model: LC – 10 ATVP) and a micro syringe of capacity 25 µl (Model: Microliter® # 702, Mfd. by: M/s Hamilton).

The mobile phase consists of a mixture of water containing 0.1% w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitirle (34: 66). The mobile phase was filtered through 0.45µm membrane filter before use and was run at a flow rate of 1ml/min.

Detection: The column effluent was monitored at 254 nm.

Estimation of celecoxib in plasma:

For the estimation of celecoxib in plasma samples, a calibration curve was constructed initially by analyzing plasma samples containing different amounts of celecoxib as follows.

To a series of tubes containing 0.5ml of plasma in each, 0.1 ml drug solution containing 1, 2, 4, and 6 μg of celecoxib were added and mixed. To each tube 1 ml of acetonitrile was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 ml) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5ml of mobile phase [a mixer of water containing 0.1% w/v sodium phosphate monobasic (pH adjusted to 2.1 using phosphoric acid) and acetonitrile (34:66)] was added and mixed for reconstitution. Subsequently 20 μl were injected into the column for HPLC analysis.

The Plasma concentration of celecoxib and the corresponding peak areas are given in Table 6.5 and shown in Fig.6.3. This calibration curve was used for the estimation of celecoxib in the plasma samples collected in the pharmacokinetic evaluation. Plasma (0.5 ml) was analyzed for celecoxib as above under calibration curve.

Table 6.5: Calibration curve for the Estimation of Celecoxib in Plasma Samples by HPLC Method

Celecoxib concentration (µg/0.5ml of plasma)	Mean Peak Area (mV.s)	RSD (%)
1.0	41.65	0.45
2.0	83.40	0.62
4.0	167.20	0.38
6.0	251.95	0.82

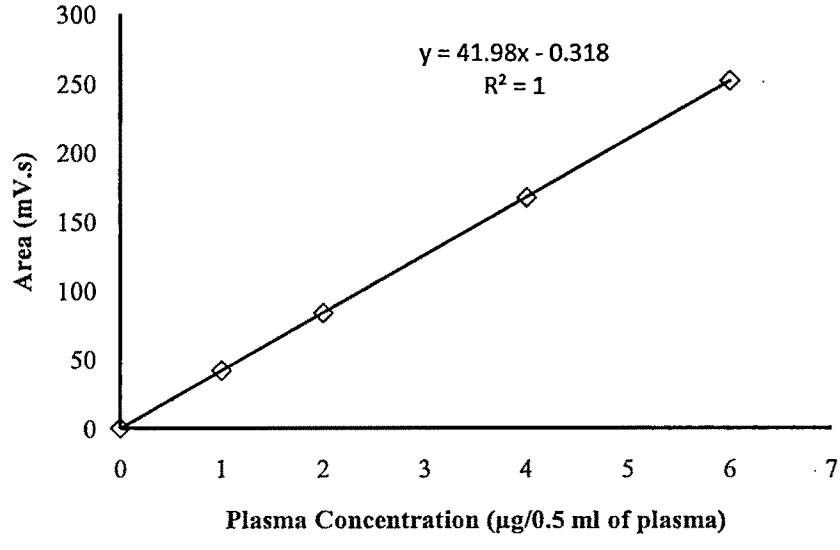

Fig.6.3: Calibration curve for the Estimation of Celecoxib in Plasma Samples by HPLC Method

ESTIMATION OF ACECLOFENAC IN PLASMA BY HPLC METHOD

A known[1] HPLC method with UV detection was used for the estimation of aceclofenac in plasma.

Instrumentation:

The HPLC system (Make: M/s Shimadzu Corporation, Japan.) consisted of UV-Visible detector (Shimadzu, Model: SPD – 10AVP), C-18 column (Phenomenex, DESC: Gemini 5µ C18 110A, Size: 250 X 4.6 mm, S/No: 288063 – 23), 2 pumps (Model: LC – 10 ATVP) and a microsyringe of capacity 25µl (Model: Microliter® # 702, Mfd.by: M/s Hamilton).

Chromatographic Conditions:

Mobile Phase:

The mobile phase was a mixture of 5 mM sodium phosphate buffer (pH 7.2) and acetonitrile (67: 33 v/v). The mobile phase was filtered through a 0.45µm membrane filter before use and was run at a flow rate of 1 ml/min.

Preparation of Standard Plasma Samples:

A 500 µl aliquot of acetonitrile was added to a 200 µl aliquot of plasma samples containing known quantities of aceclofenac. After vigorous mixing, the mixture was centrifuged at 6000 rpm for 2 minutes. A 100 µl aliquot of the supernatant was injected directly on to the HPLC column. The mobile phase was run at a flow rate of 1 ml/min.

Detection: The column effluent was monitored at 280nm.

The concentrations of aceclofenac and the corresponding peak areas are given in Table 6.6. The concentrations of aceclofenac were plotted against peak areas as shown in Fig 6.4

Table 6.6

Calibration Curve for the Estimation of Aceclofenac in Plasma

Aceclofenac Concentration (µg / 0.5 ml of plasma)	Mean Peak Area (mV.s)	RSD (%)
1	56.42	0.65
2	113.32	0.28
4	230.54	0.46
6	351.20	0.85

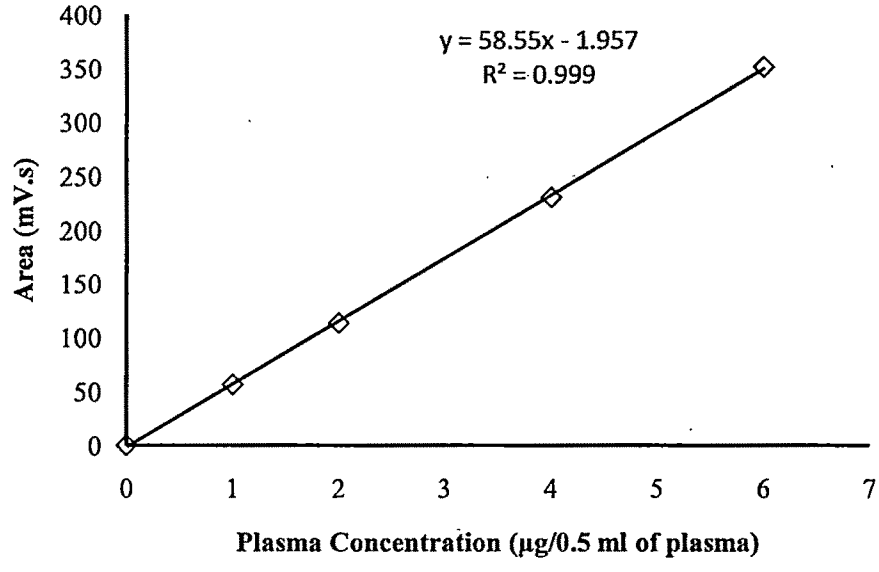

Fig 6.4

Calibration Curve for the Estimation of Aceclofenac by HPLC Method

REFERENCE

1. Kin, Y.G., Lee, Y.J. and Kim, H.J., **Int. J. of Clin. Pharmacol. And Ther.,** 39, 83, 2001.