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UV spectrophotometric method for the quantitative estimation of celecoxib in capsule dosage forms

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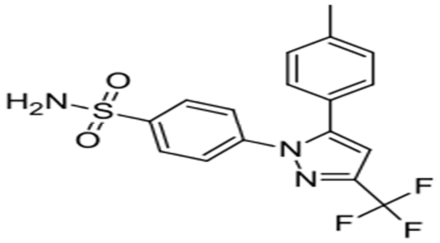
ABSTRACT

A simple UV Spectrophotometric method for the quantitative estimation of Celecoxib using analytical grade methanol as solvent and acetyl chloride as the reagent for acetylation of Celecoxib has been developed. Acetyl derivative of Celecoxib obeys Beer's law in concentration range 20-40 µg/ml at 270nm as absorption maximum. The recovery studies ascertained accuracy of purposed method and result validated according to ICH guidelines. The result of analysis has been validated statistically by recovery studies. This method was successfully carried out for the estimation of Celecoxib in capsule dosage form without the interference of common excipients.

Keywords: Celecoxib, Area under curve method, Spectrophotometric method, UV determination.

INTRODUCTION



Celecoxib

Celecoxib is 4-[5-(4-Methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzenesulfonamide prescribed in osteoarthritis, rheumatoid arthritis, acute pain, musculoskeletal pain, ankylosing spondylitis, painful menstruation and to reduce the number of colon and rectal polyps in people with familial adenomatous polyposis. Tentative evidence indicates its use in treatment of psychiatric disorders, including major depression, bipolar disorder, and schizophrenia. It has been used to overcome colon and rectal polyps in people with familial adenomatous polyposis [Internet] There are twelve methods have been reported for the estimation of Celecoxib in pharmaceutical formulations, which include nine HPLC methods [2 to 11], one Area under curve method [12] and three UV Spectrophotometric methods [2, 13 and 14]. Aim of the present study, there for; was to develop a simple UV Spectrophotometric method, which can be alternative to HPLC and zero order UV Spectrophotometric methods.

MATERIALS AND METHODS

Materials:
Shimadzu 1800 spectronic UV Spectrophotometer with 1cm matched quartz cells was used for data collection and analysis. Methanol (95%) and Acetyl Chloride were used as a solvent and reagent for drug substance.

Methodology:
Preparation of standard stock solution:
Standard stock solution of celecoxib was prepared by dissolving accurately weighed quantity of celecoxib 25mg in 25 ml of methanol and transferred it to 25 ml of volumetric flask. Volume was created to the mark with methanol for obtaining stock solution up to 1000µg/ml conc. Further dilution made to get the concentration of 100µg/ml.

Determination of Absorption Maximum:
The standard solution of Celecoxib (10µg/ml) was scanned in the wavelength from 230 to 280 and absorption maximum was found to be 252nm. (Figure 1)

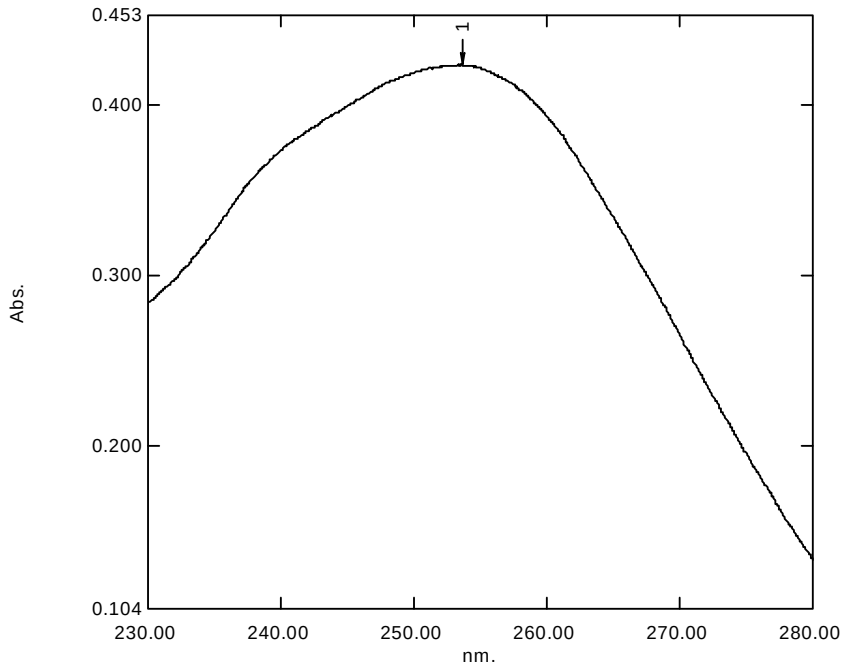


Figure 1: Absorption maximum of Celecoxib in methanol

Determination of Absorption maximum of Acetyl Derivative:
10ml from the stock solution of 100µg/ml Celecoxib were pipette to three different volumetric flasks. In the first volumetric flask, acetyl chloride solution 2%, 5ml was added. In the second volumetric flask, acetyl chloride 5%, 5ml was added. To the third volumetric flask, 5%, 10ml acetyl chloride was added. All the solutions were diluted to 100ml volume using methanol 95% to get a final concentration of 10µg/ml Celecoxib and the spectra were recorded. The absorption maximum was found to be at 270nm and the reagent concentration of 5% acetyl chloride with 5ml volume showed maximum intensity at 270nm, selected as the analytical wavelength for the determination of Celecoxib. (Figures 2, 3 and 4)

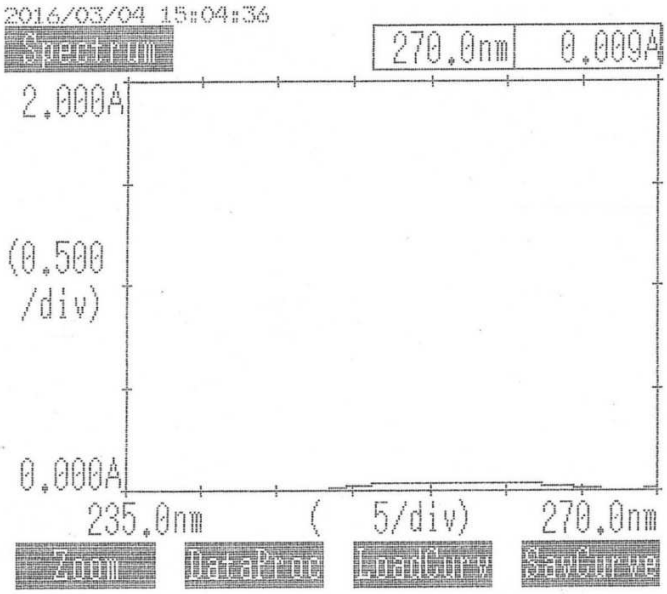


Figure 2: 10µg/ml Celecoxib solution with 2%, 5ml Acetyl Chloride

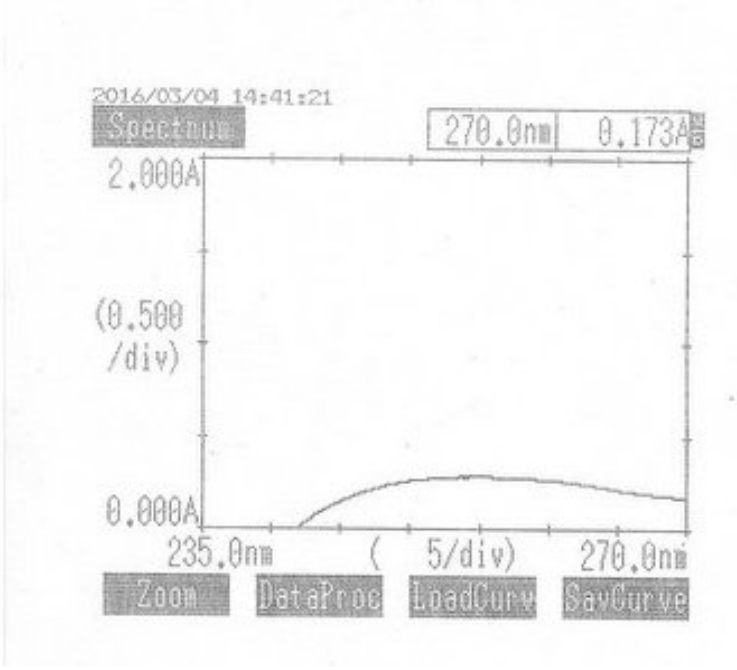


Figure 3: 10µg/ml Celecoxib solution with 5%, 5ml Acetyl Chloride

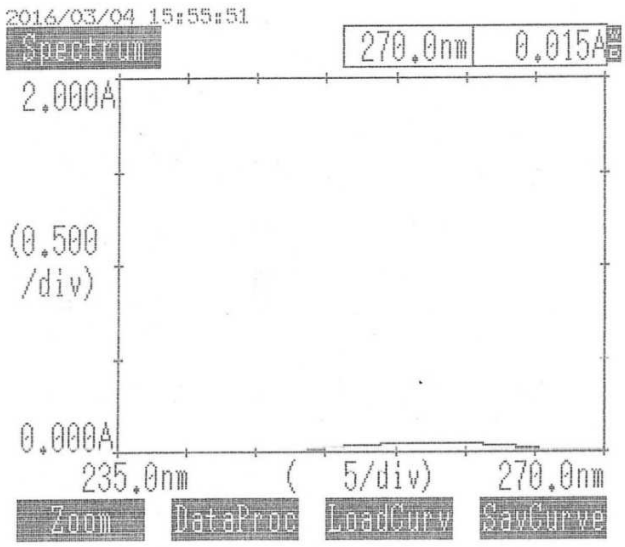


Figure 4: 10µg/ml Celecoxib solution with 5%, 10ml Acetyl Chloride

Stability of Drug in Selected Solvent and Reagent:

The stability of drug in selected solvent was determined by measuring the absorbance of the drug solution (20µg/ml) at different time intervals. After every 5 min. of interval the abs. was measured the solution was found to be stable. (Table 1)

Table 1: Stability Data for Celecoxib

Sr. No.	Time(min.)	Absorbance
1	0	0.279
2	05	0.279
3	10	0.281
4	15	0.279
5	20	0.278
6	25	0.277
7	30	0.277

Linearity:

From the standard stock solution of Celecoxib, appropriate aliquots were pipette out into 25 ml of volumetric flask, 5%, 5ml acetyl chloride solution was added and dilutions were made with methanol to produce working standard solution of Celecoxib 20,25,30,35 and 40µg/ml concentrations. The absorbances of Celecoxib solutions were measured at 270nm. The calibration plot of the drug Celecoxib was plotted.

The concentration range over which the drug followed linearity was chosen as an analytical concentration range i.e. 10 to 50µg/ml for Celecoxib. (Table 2 and Figures 5 to 10)

Table 2: Standard Calibration Table for Celecoxib

Sr. No.	Conc.(µg/ml)	Absorbance
1.	20	0.055
2.	25	0.102
3.	30	0.184
4.	35	0.262
5.	40	0.337
Regression Equation:	y = 0.01448x – 0.2464	Regression Coefficient: r^2 = 0.9965

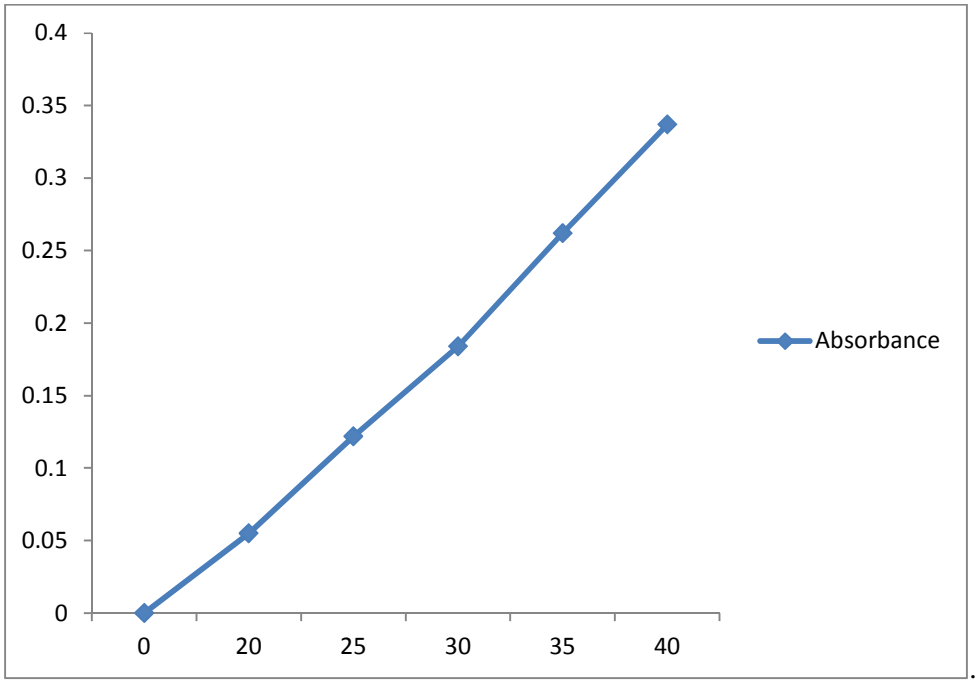


Figure 5: Calibration Plot for Linearity

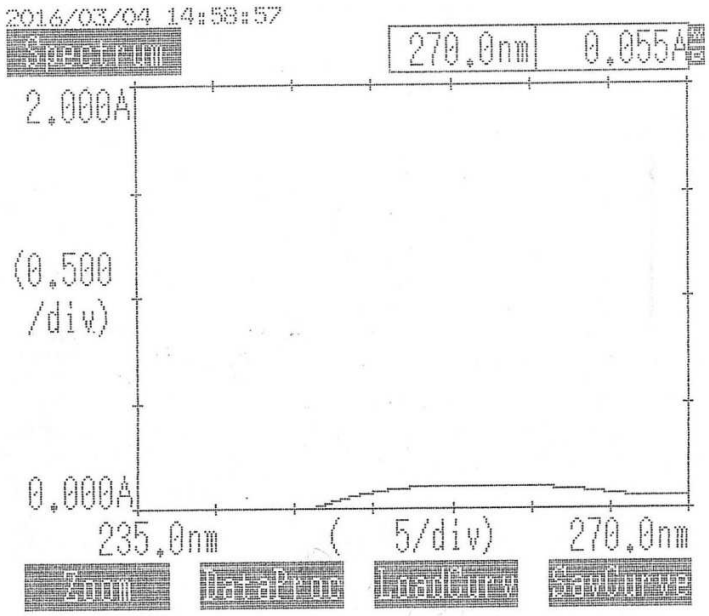


Figure 6: Celecoxib Concentration 20µg/ml

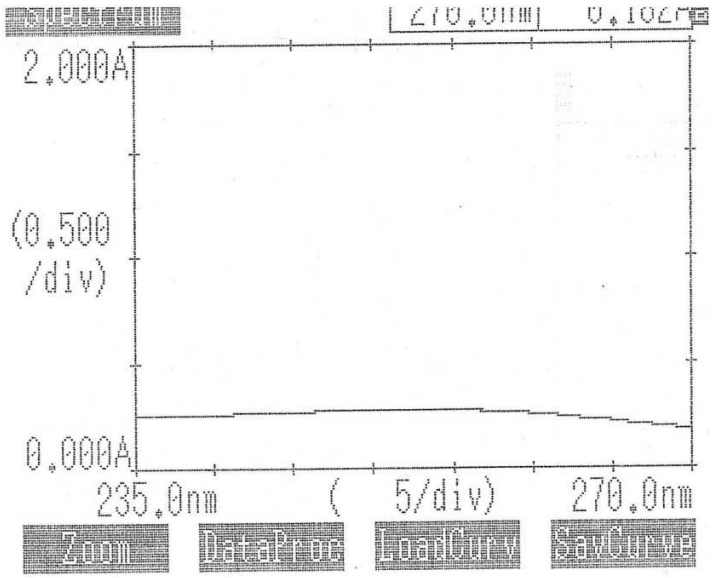


Figure 7: Celecoxib Concentration 25µg/ml

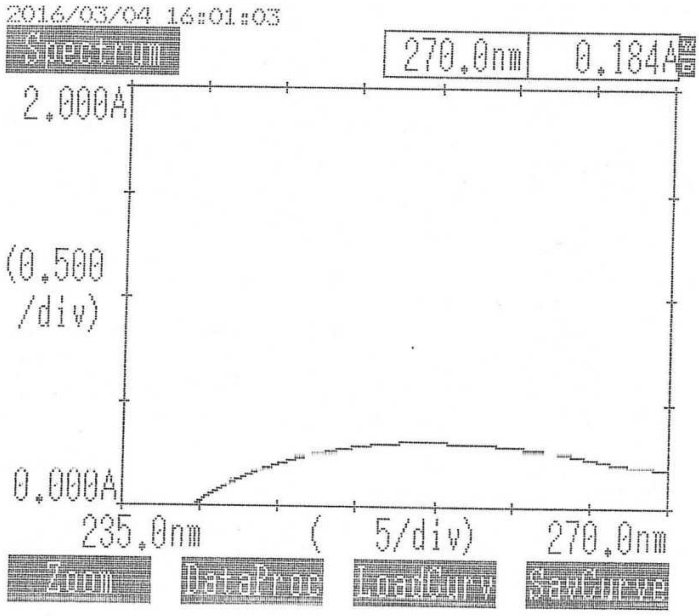


Figure 8: Celecoxib Concentration 30µg/ml

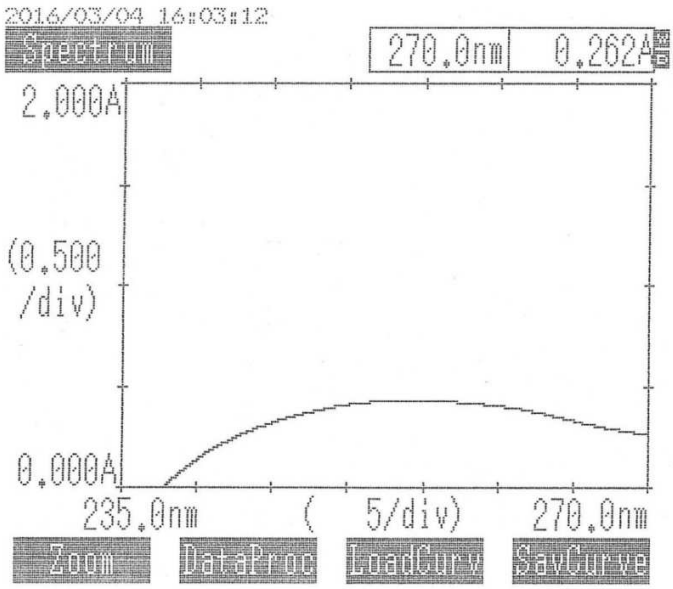


Figure 9: Celecoxib Concentration 35µg/ml

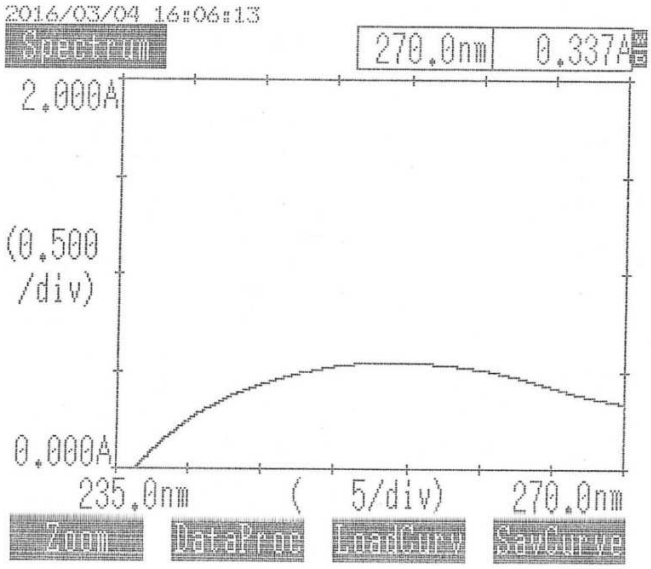


Figure 10: Celecoxib Concentration 40µg/ml

Validation of purposed method:

A. Estimation of Drug from Dosage Form (capsule): (Assay Study)

Brand name- Celedol and Zycel

Standard:

From the standard stock solution of Celecoxib, appropriate aliquots were pipette out into 25 ml volumetric flask, 5%, 5ml acetyl chloride solution was added and dilutions were made with methanol to obtain working standard solution of Celecoxib 20µg/ml. Absorbance of this concentration was taken at 270nm absorption maximum.

Sample:
Twenty capsule contents of brand Celedol (Ipca Laboratories) and Zycel (Zydus healthcare) containing 200 mg of celecoxib weighed, and finally powered. A quantity of powder sample of 31mg from Celedol and 46mg from Zycel was taken into different volumetric flask, 5%, 5ml acetyl chloride was added. Dilutions were made to get concentration of 20µg/ml. for both brands. These concentrations were scanned at 270nm. (Table 3)

Table 3: Assay for Celecoxib as Celedol and Zycel Capsule Formulations

Brand Name	Label Claim (mg/capsule)	Amount Found (mg/capsule)	% Of Label claim	Mean	SD	CV
Celedol	200	199.53	99.76	100.064	0.2387	0.05698
	200	200.46	100.23			
	200	200.75	100.37			
	200	199.98	99.99			
	200	199.95	99.97			
Zycel	200	199.23	99.61	99.84	0.2458	0.06042
	200	200.07	100.03			
	200	200.03	100.01			
	200	200.08	100.04			
	200	199.10	99.55			

B. Accuracy (Recovery Study):
Recovery experiments are used for study of accuracy method. This study was carried out by adding known amount bulk sample to capsule, and recovery was performed at three levels, 80, 100 and 120% of celecoxib standard concentration with acetyl chloride reagent. Samples for recovery studies were prepared according to aforementioned procedure. 3 samples were prepared for each recovery level. The solutions of sample were analyzed, and % recoveries were calculated by using fallowing formula.

$$\%\,Recovery = \frac{observedamountofcompoundinsample}{Amountofallcompoundpresentinsample} \times 100$$

The recovery values are summarized in following tables 4 and 5.

Table 4: Accuracy parameters of Celecoxib (Brand Celedol)

Label % recovery	*Amount present (mg/capsule)	Amount Of Standard added (mg/capsule)	Total Amount Recovered (mg/capsule)	% recovery	%mean recovery	SD	CV
80	200	160	159.94	99.63	99.53	0.3108	0.09663
80	200	160	160.4	100.25			
80	200	160	159.82	99.88			
100	200	200	200.09	100.45	99.90	0.4726	0.2234
100	200	200	199.36	99.68			
100	200	200	199.18	99.59			
120	200	240	241.63	100.68	100.21	0.4803	0.2307
120	200	240	240.55	100.23			
120	200	240	239.32	99.72			

Table 5: Accuracy parameters of Celecoxib (Brand Zycel)

Label % recovery	*Amount present (mg/capsule)	Amount of Standard added (mg/capsule)	Total Amount Recovered (mg/capsule)	% recovery	%mean recover	SD	CV
80	200	160	158.32	98.95	99.45	0.6285	0.3950
80	200	160	160.25	100.16			
80	200	160	158.81	99.26			
100	200	200	199.54	99.77	99.93	0.1600	0.0256
100	200	200	199.86	99.93			
100	200	200	200.18	100.09			
120	200	240	240.45	100.19	100.023	0.1700	0.0289
120	200	240	239.64	99.85			
120	200	240	240.07	100.03			

C. Precision:

Four independent sample of celecoxib are used for evaluation of precision. The intermediate precision (interday precision) of the method was also evaluated using 4 different analysts in the same laboratory. The values obtained by four analysts were summarized in table 6.

Table 6: Determination of Precision of Celecoxib

Sample Number	Assay of Celecoxib as % of Labeled amount(inter – day precision)			
	Analyst 1	Analyst 2	Analyst 3	Analyst 4
1	99.86	100.35	99.97	100.13
2	99.53	99.82	100.02	99.74
3	100.05	99.95	100.05	99.46
4	99.85	99.82	100.09	100.12
Mean	99.82	99.98	100.03	99.86
SD	0.2156	0.2509	0.05058	0.3239
CV	0.04649	0.06296	0.00255	0.1049

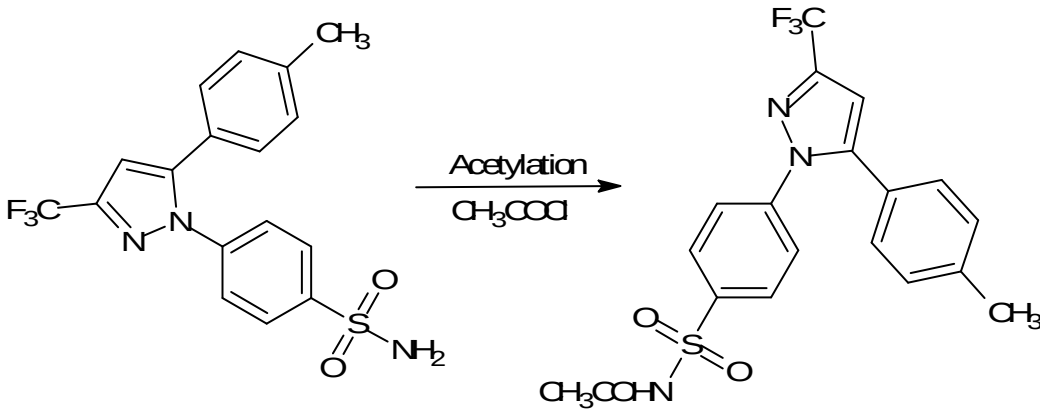
RESULTS

The standard solutions of Celecoxib in Methanol with 5%, 5ml Acetyl chloride reagent (20µg/ml each) subjected to scanning at 235 to 300nm and the absorption maxima was found to be 270nm using Shimadzu 1800 spectronic UV-Visible spectrophotometer. The calibration curve of Celecoxib was found to be linear at conc. Range 20 to 40 µg/ml at this absorption maximum. There for, it was clear that Celecoxib can be determined in presence of methanol with no intervention of any irrelevant substance in pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands (Celedol and Zycel) of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample as well as concentrations were estimated. Then the technique was subjected to the assay of in marketed dosage forms and satisfactory results were attained within the appropriate limits as per the content of the label claim for Celecoxib. The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Celecoxib was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through inter day precision studies, where the samples were subjected to changed conditions other than optimized parameters.

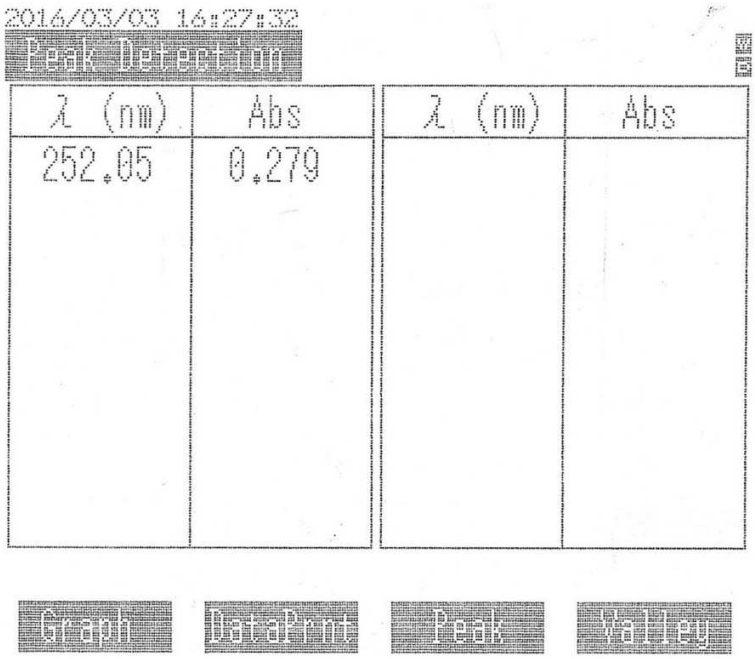
DISCUSSION

Amino (NH_2) group of Celecoxib reacts with acetyl chloride to give acetyl derivative, which shifts the Lambda max (absorption maximum) of Celecoxib towards higher wavelength (bathochromic shift) i.e. from 252nm to 270nm.



After The Addition of Acetyl Chloride The λ Max Is Increases from 252 nm to 270nm. 10µg/ml Celecoxib with 2%, 5ml Solution of Acetyl Chloride shows absorbance maxima of 270nm and absorbance of 0.009 (low intensity). 10µg/ml Celecoxib with 5%, 5ml Solution of Acetyl Chloride shows absorbance maxima of 270nm and absorbance of 0.173 (increased intensity). 10µg/ml Celecoxib with 5%, 10ml Solution of Acetyl Chloride shows absorbance maxima of 253nm and absorbance of 0.015 (decreased intensity). Therefor, a reagent concentration of 5% and reagent volume of 5ml was selected and 270nm was selected as analytical wavelength for the determination of Celecoxib in bulk drugs. (Table 7 and 8)

Table 7: Absorption Maximum of Celecoxib in Methanol 252nm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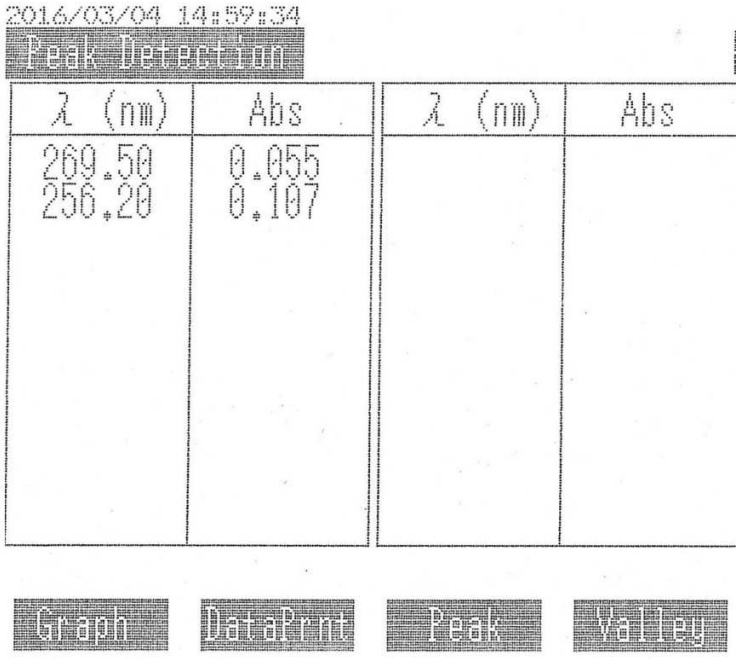
Peak Detection

λ (nm)	Abs	λ (nm)	Abs
252.05	0.279		

Graph DataPoint Peak Valley

Table 8: Absorption Maximum of Acetyl Derivative of Celecoxib 270nm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Peak Detection

λ (nm)	Abs	λ (nm)	Abs
269.50	0.055		
256.20	0.107		

Graph DataPoint Peak Valley

CONCLUSION

From the above experimental studies it is concluded that area under curve method developed for estimation of Celecoxib was suitable for the routine determination of Celecoxib. The proposed method for the selected drug Celecoxib was found to be precise and accurate. The most important striking features of spectrophotometric methods are their rapidity and simplicity. The newly developed method is alternative to HPLC methods and zero order UV spectrophotometric methods. Results of validation parameters demonstrate that these performed analytical procedures are suitable for its intended purpose and meet the criteria defined in ICHQ2A/B guidelines.

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