Spectrophotometric Determination of Celecoxib in Pharmaceutical Preparations

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ABSTRACT

Two simple and sensitive spectrophotometric methods have been developed for the estimation of Celecoxib in pure and pharmaceutical dosage forms. Method A is based on ion association complex formation of the drug with Methylene Blue . Method B is based on the charge transfer complexation reaction between Celecoxib and iodine. The absorption spectrum of Celecoxib – methylene blue and Celecoxib –iodine reaction products showed absorption peaks at 665 and 511 nm respectively. The systems obeyed the Beer's law in the range 0.2–1μg/ml for both the methods A and B. The correlation coefficients and the limits of assays detection values were found to be 0.999, 0.37 for (method A) and 0.998, 0.426 for (method B). , respectively. Proposed methods were successfully applied to the quantitative determination of celecoxib in pharmaceutical dosage forms with good accuracy.

Introduction

Celecoxib is a sulfa non-steroidal anti-inflammatory drug (NSAID) and selective COX-2 inhibitor used in the treatment of osteoarthritis, rheumatoid arthritis ,acute pain ,painful menstruation and menstrual symptoms, and to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis[1]. Celecoxib is licensed for use in osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation and menstrual symptoms, ankylosing spondylitis and to reduce the number of colon and rectal polyps in patients with familial adenomatous polyposis[2,3]. It was originally intended to relieve pain while minimizing the gastrointestinal adverse effects usually seen with conventional NSAIDs. In practice, its primary indication is in patients who need regular and long term pain relief: there is probably no advantage to using celecoxib for short term or acute pain relief over conventional NSAIDs, except in the situation where non-selective NSAIDs or aspirin cause cutaneous reactions[4-6]. In the literature, different analytical methods have been reported for analysis of COX-2 inhibitors. An HPLC method with UV and MS detection has been developed for assay of some COX-2 inhibitors in biological samples and pharmaceutical preparations [7–14]. The objective of the work described in

this paper was to quantitative analysis of celecoxib in pharmaceutical preparations by ion association complex and charge transfer complex reactions .

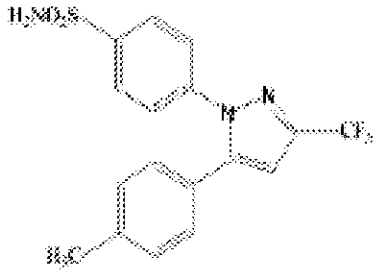


Fig. 1. The chemical structure of Celecoxib.

Experimental

Apparatus

1-UV –VIS spectrophotometer Shimadzu 160 Double beam

2- pH meter Kent

Reagents and Chemicals

All chemicals used were of analytical or pharmaceutical grade . Celecoxib was obtained as a gift sample from Searle Ltd., Pharmacia Corporation , U.S.A. An aqueous solution of Methylene Blue (1.563×10^{-4} M) and Iodine solution (0.003 N) was used.

Celecoxib Stock Solution

A stock solution (2mg/ml) of Celecoxib was prepared by dissolving 200 mg of the sample and the reference material, in 100 ml of methanol, The decision for using a 100% methanol was based on its sensitivity, stability and preparation time. A portion of this stock solution was diluted step wise to get the working standard solution of concentration 100µg/ml. From this solution was prepared a solution containing 20 µg/ ml, and the absorbance was measured. The solvent used in the solubilization was also used as a blank. The spectrum of Celecoxib in methanol is shown in Fig.(5). A maximum absorption at 252 was found.

Method A:

An aliquot solutions containing 0.2 – 10 µg /ml of Celecoxib were transferred into a series of 10 ml volumetric flasks . 2 ml of 1.563×10^{-4} M Methylene blue was added to each of the volumetric flasks and diluted up to the mark with methanol .The contents were shaken well and left at room temperature for 5 min , the absorbance of the blue colored complexes were measured at 665 nm against the corresponding reagent blank . A calibration graphs was plotted .Fig.(9).

Method B :

An aliquot solutions containing 0.2 –10 µg /ml of Celecoxib and 3 ml of 0.003% iodine solution were placed separately in a series of 125 ml separating funnels. The total volume in each funnel was adjusted to 10 ml with chloroform and the contents were shaken for 2 min and allowed to separate for10 min .The organic layer was collected into a 10 ml volumetric flask. The combined extract was made up to the mark with chloroform and mixed well. Absorbance of the organic layer was measured at 511 nm against the reagent blank. A calibration graphs was plotted .Fig.(10).

Assay of Celecoxib in pharmaceutical formulations:

Five tablets of Celecoxib were weighed and powdered .The average weight of a tablet was calculated. An amount of the powder equivalent to 200 mg of Celecoxib was weighed into a 100 ml volumetric flask containing about 75 ml of methanol . It was shaken well for about 20 min , filtered through a Whatman filter paper No .40 to remove the insoluble matter and diluted to the mark with methanol. A suitable aliquot of this solution in the working range of Celecoxib was treated as per procedures described above. The content of Celecoxib in the tablets was calculated from a previously plotted calibration graph .Table (5,6).

Determination Stoichiometry of complex :-

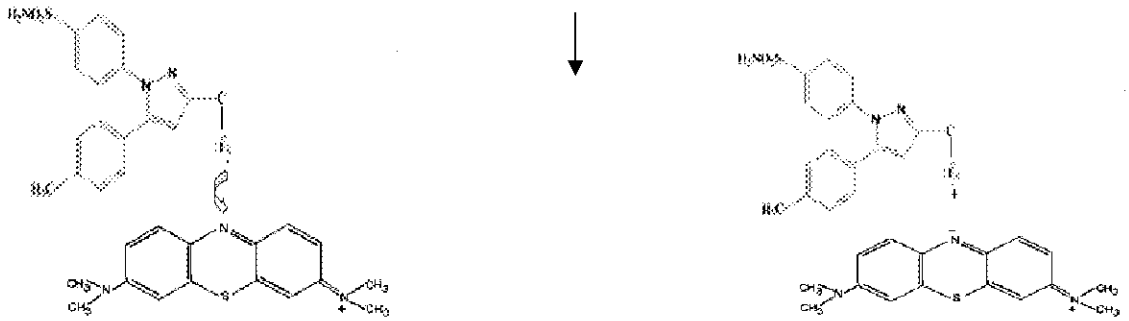
Job's method of continuous variations [15]was used to determine the nature of complex . In this method a series of mixtures are prepared which two constituents are present at varying concentrations ,but their sum is held constant . 1.563×10^{-4} M of Methylene blue ,0.003Nof Iodine and 250µg/ml of Celecoxib were prepared. To seven 125 ml separation funnels ,2 , 3, 4 , 5 ,6 , 7, and 8 ml of Celecoxib solution were added and then 8 , 7 ,6 ,5 , 4 ,3, and 2 ml of methylene blue were transferred .mixed well , the reaction was allowed to proceed to equilibrium at least 5 min., the absorbance of the mixtures were measured at 665 nm . To seven 125 ml separating funnels 2 , 3, 4 , 5 ,6 , 7, and 8 ml of Celecoxib solution were added and then 8 , 7 ,6 ,5 , 4 ,3, and 2 ml of iodine were transferred ,the contents were shaken for 2 min and allowed to separate for10 min .The organic layer was collected into a 10 ml volumetric flask. The combined extract was made up to the mark with chloroform and mixed well. The reaction was allowed to proceed to equilibrium at least 5 min . the absorbance of the mixtures were measured at 665 nm for methylene blue and 511 nm for iodine .Normally , a maximum appear in the curve at a mole fraction corresponding to the complex that forms . Fig.(11,12) .

Results and discussion

The solution spectra of Methylene blue in visible region exhibited two bands at 668 and 609 nm , this bands due to π -π^* transitions, Fig.(4). Drug showed negligible absorption in 300–700 nm and showed absorption at 252 nm, Fig.(5). The results obtained in method A involves an ion association complex

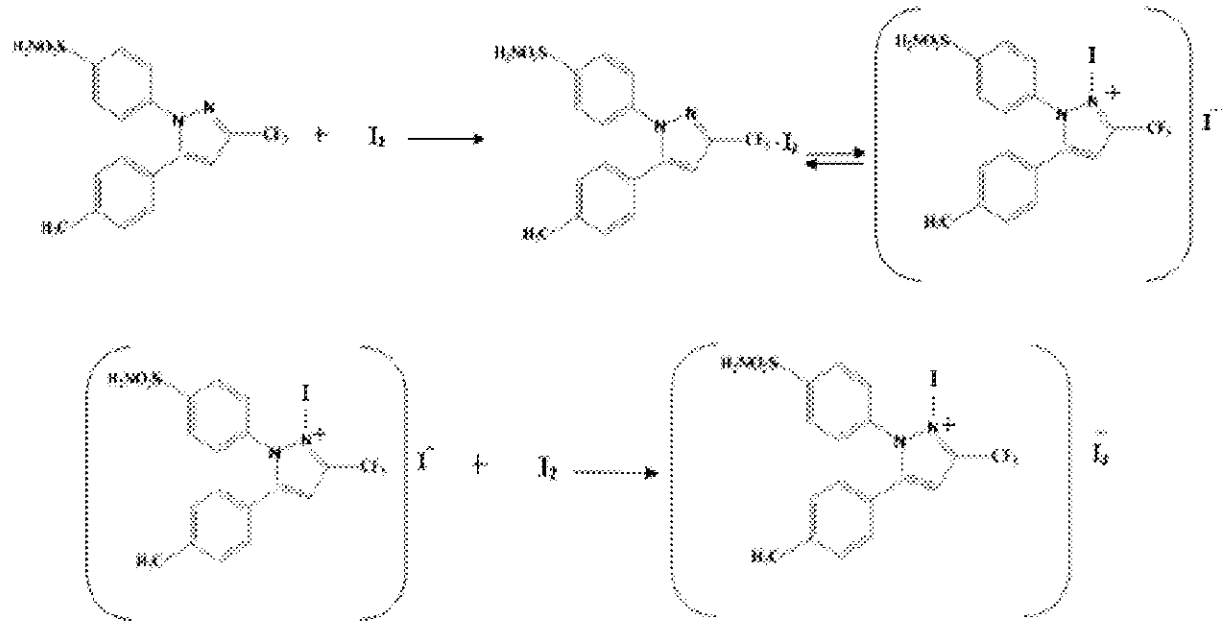
formation with Methylene blue to give a blue color solution . The absorption spectrum of Celecoxib – methylene blue reaction product showed absorption peak at 665 nm , Fig.(6). The results obtained in method B were based on the charge transfer reaction . In Charge – transfer complexs, one species acts as an electron donor and another as an electron acceptor. Transition of an electron from an orbital of the donor species to an orbital of the acceptor can result from absorption of radiation .The absorption spectrum of iodine in chloroform showed two peaks with maximum absorption at 513 and 287 nm ,Fig.(7). Experimental drug was basic nitrogen compound , which act as n–donors to form CT complex with iodine (σ acceptor). Donor was completely transparent to visible light when mixed with chloroformic solution of iodine resulted in the change of violet color of iodine to rose color . This due to charge transfer complexation reaction between Celecoxib and iodine . The absorption spectrum of Celecoxib – iodine reaction product showed absorption peak at 511 nm, a new peak at 374 nm was observed , it can be attribute to charge transfer reaction , Fig.(8) The structure of the ion association complex and charge transfer complex are shown in , Fig. (2,3), respectively .

Methylene Blue
Celecoxib



Ion Association Complex

Fig .2. Reaction Scheme



Tri- iodide ion pair of Charge Transfer Complex

Fig .3. Reaction Scheme

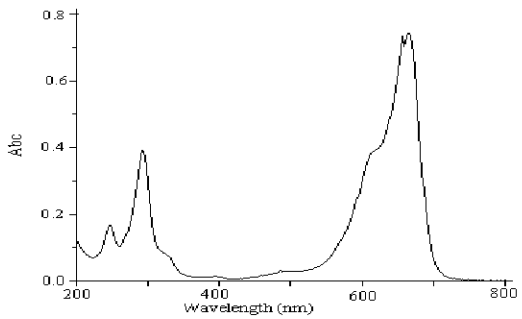


Fig .4. Spectrum of methylene blue

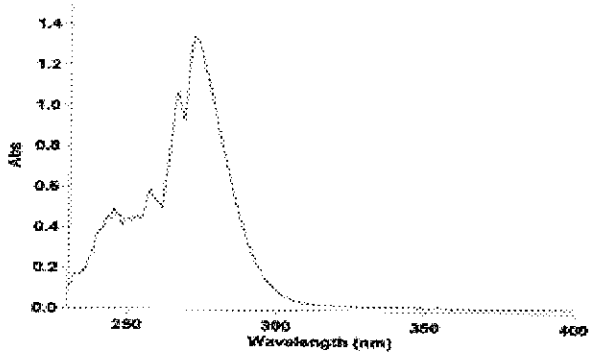


Fig. 5. The ultraviolet spectra of Celecoxib

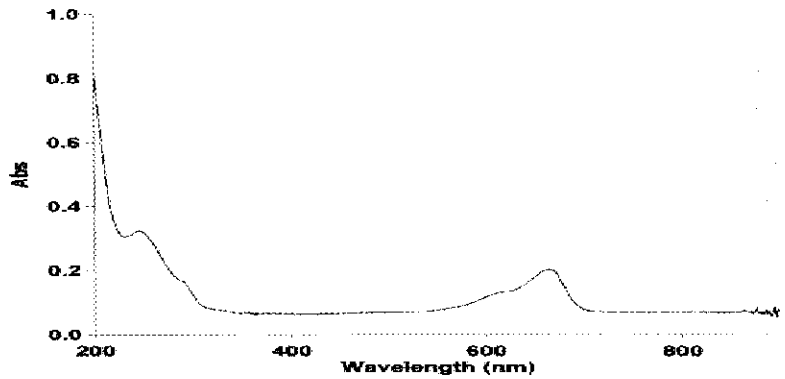


Fig.6 . Absorption Spectrum of Ion association Complex of Celecoxib and methylene blue

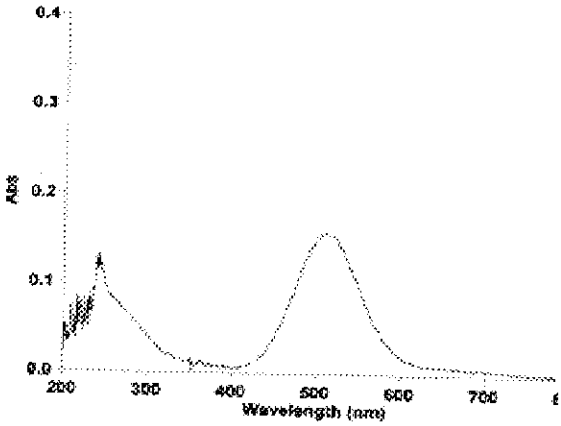


Fig.7. Spectrum of iodine

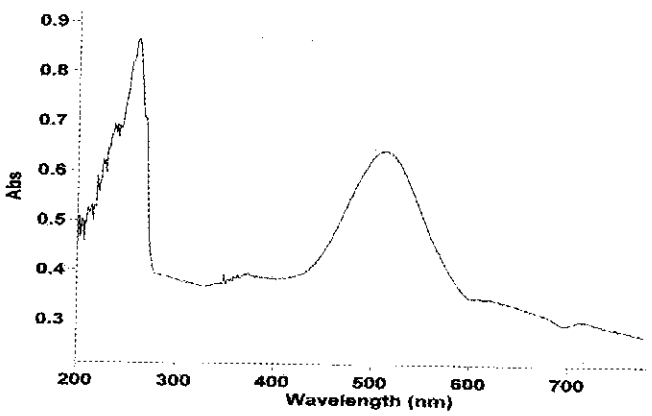


Fig.8. Absorption Spectrum of Charge Transfer Complex of Celecoxib and Iodine

Linearity and Range

Beer's law range , molar absorptivity , regression equation and correlation coefficient determined are given in Table(3). Linear relationships were found between the absorbance at λ_{max} and the concentration of the drug in the range 0.2 –10 µg /ml .Regression analysis of Beer's plots at λ_{max} reveals a good correlation . The graphs show low intercept and is described by the regression equation obtained by the least squares method , Fig.(9,10).The correlation coefficient was 0.999 ,0.998 indicating good linearity, and the high molar absorptivities (7.45×10^3 l mol^{-1} cm^{-1} , 9.59×10^3 l mol^{-1} cm^{-1}) of the resulting coloured complex indicate the high sensitivity of the method . The limits of detection (LOD) and limits of quantitation (LOQ) were (0.37 ,0.14)for method A and (0.426,0.29)µg /ml for method B respectively . These low values of LOD and LOQ confirmed the high sensitivity of the methods and consequently.

Table(3) Optical Characteristics , Precision and Accuracy Data

Parameter	Proposed methods	
	Method (A)	Method (B)
λ_{max} (nm)	665	511
Molar absorptivity(lmol^{-1} cm^{-1})	7.45×10^3	9.59×10^3
Beer's law (µg/ml)	0.2–10	0.2.–10
Linear Regression equation,Y^a	$Y = 2.1267X - 0.293$	$Y=1.972\,X-0.406$
Slope (b)	2.126	1.972
Intercept(c)	–0.293	–0.406
Correlation Coefficient ,R	0.999	0.998
Limit of detection (µg/ml)	0.37	0.426
Limit of quantification (µg/ml)	0.14	0.29

a) $Y= bx+c$, were x is the concentration of drug in µg/ml

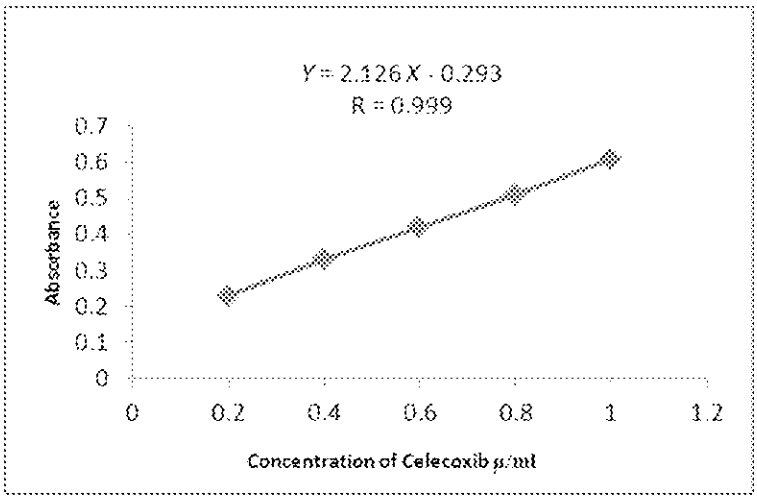


Fig.9. Calibration graph of Complex with methylene blue

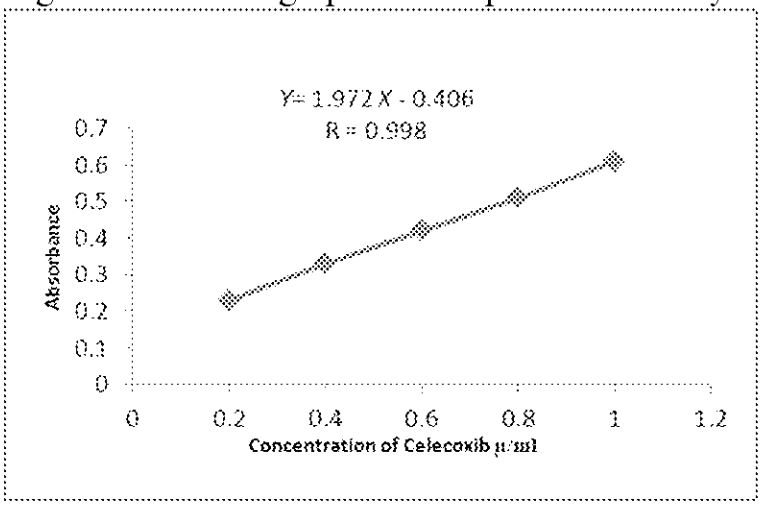


Fig .10.Calibration graph of Complex with Iodine

Stoichiometry of complex :-

The drug to both dye and iodine stoichiometric ratio were found to be a 1:1 complexes, Fig.(11,12) .

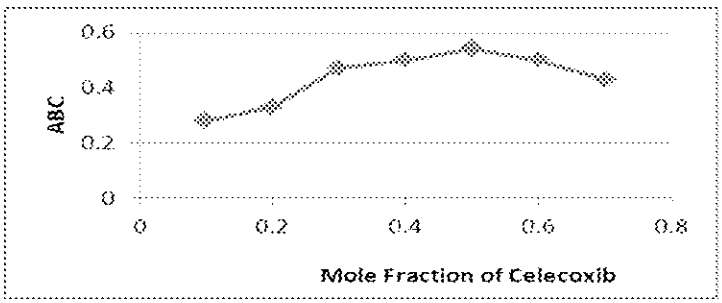


Fig .11 . Stoichiometry of complex with metylene blue

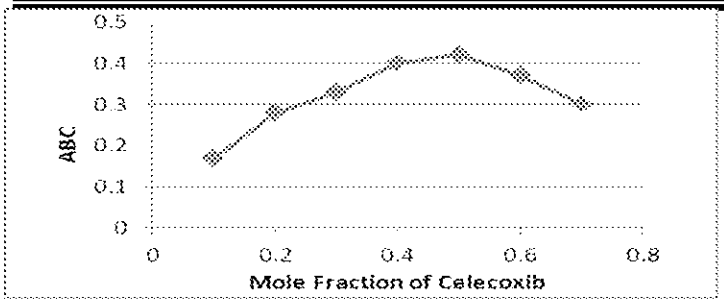


Fig .12 . Stoichiometry of complex with Iodine

Optimization of Reaction Conditions

Optimum reaction conditions for quantitative determination of ion association complex and charge –transfer complex were established via a number of preliminary experiments .

Effect of pH

Celecoxib is a weak acid ,the acidic nature of celecoxib is due to the presence of sulphonamide group, the pH of the complexes were 5.5 for method A and 4.7 for method B . To study the effect of pH, 20 µg mL^{-1} of the drug was used and the reactions were carried at different range of pH from 2-10 . The results show that the maximum value of absorption was at pH 5.5 for method A and at pH 4.7 for method B respectively and that is mean , there is no need to control the acidity of reaction ,Fig.(13,14).

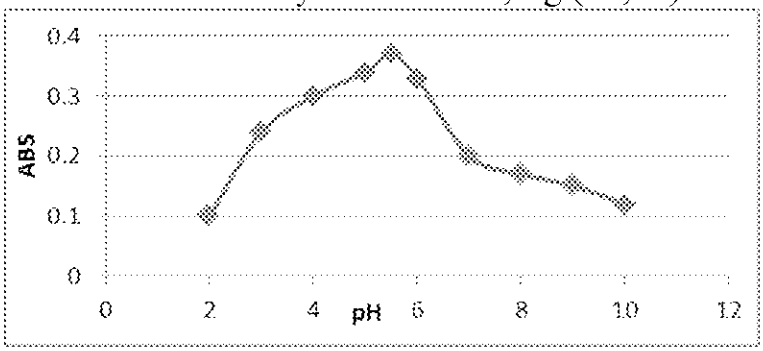


Fig.13. The effect of pH on ion association formation of Celecoxib, 20 µg mL^{-1} with methylene blue at 665nm.

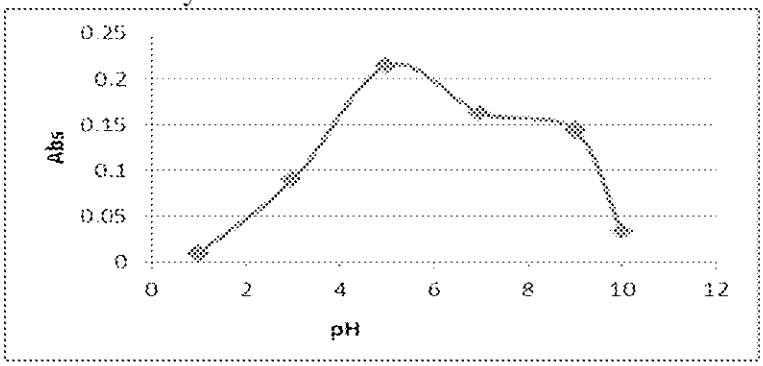


Fig.14. The effect of pH on charge transfer formation of Celecoxib, 20 µg mL^{-1} with iodine at 511 nm.

Effect of Temperature

To study the effect of temperature on reaction yield, we carried out the reaction at 25 , 30 , and 40 C^0 . The obtained results showed that the reaction of drug with methylene blue and iodine were completed at room temperature and there was no need heating the reaction solution .The complexes were stable for 24 h at room temperature .

Effect of Reagent Concentration

The optimum reagent concentration was determined by adding various volumes of the methylene dye and iodine to a fixed concentration of 20 µg mL^{-1} of the drugs. It was found that 2 ml of 1.563×10^{-4} M Methylene blue and 3 ml of 0.003% iodine ciprofloxacin were enough to develop the absorbance to its maximum intensity .

Effect of Time

The coloured of complexes with methylene blue and iodine developed after the reagents had been added and mixed attained maximum intensity after about 5 min and 10 min at $25C^0$ for methylene blue and iodine respectively .

Quantitation of Celecoxib in pharmaceutical preparation:-

The Celecoxib concentration in each tablet was quantified by measuring the absorbance of the complex for each tablet solution and compare the result with the calibration curve of the standard solutions. Table (4) summarize the result .

Table (4) . RSD% , Recovery , and E% of Celecoxib samples.

Values are expressed as means (n= 5).

Number of Samples	Labeled Amount(mg)	Determind Amount(mg)	S .D	RSD%	Recovery%	E%
n= 5	200	199.7	0.212	0.106	99.85	0.15

Ruggedness

To ascertain the ruggedness of the methods , five replicate determination at different concentration levels of the drugs were carried out . The values of RSD% for different concentrations of drug from the determination are given in Table(5,6) , and indicated that the proposed method has reasonable ruggedness.

Table (5) . Determination of Celecoxib in Pharmaceutical Preparations(method A) .

Sample No.	Clecoxib Taken (µg /ml)[c]	The proposed method Found (µg/ml)[d]	S.D	RSD%	E%	Recovery%
1	5	4.8	0.141	2.877	4	96
2	10	10.4	0.282	2.764	– 4	104
3	15	14.7	0.212	1.427	2	98
4	20	20.3	0.212	1.052	–1.5	101.5
5	25	24.75	0.53	2.538	1	99.2

a) Calculated with respect to the total weight

b) Found by using methylene blue

Table (6) . Determination of Clecoxib in Pharmaceuticals Preparations (method B).

Sample No.	Clecoxib Taken (µg /ml)[c]	The proposed method Found (µg/ml)[d]	S.D	RS%	E%	Recovery
1	5	5.3	0.212	4.116	–6	106
2	10	10.2	0.141	1.396	–2	102
3	15	14.5	0.353	2.393	3.333	96.666
4	20	20.7	0.494	2.427	–3.5	103.5
5	25	24.35	0.459	1.86	2.6	97.4

c) Calculated with respect to the total weight

*d)*Found by using iodine

Conclusion

The proposed methods for the estimation of Celecoxib were simple, sensitive and accurate and can be used for determination the drug in tablet dosage form . Hence it can adopted for routine analysis of the drug in pharmaceutical formulations.

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تقدير السليكوكسب طيفيا في المستحضرات الصيدلانية

لمياء لفتة غشيم
قسم الكيمياء/كلية العلوم/ الجامعة المستنصرية

الخلاصة

تضمن البحث طريقتين لتقدير العقار الطبي السليكوكسب تميزت بالبساطة والحساسية . الطريقة (ا) تعتمد على تكوين معقد تجمع ايوني بين الدواء و صبغة المثيلين الزرقاء، الطريقة (ب) تعتمد على تكوين معقد انتقال شحنة بين الدواء واليود. طيف الامتصاص الناتج من تفاعل الدواء مع صبغة المثيلن الزرقاء و اليود اظهر حزم امتصاص عند الطول الموجي 665 و 511 نانوميتر على التوالي. لوحظ ان طريقة التقدير تخضع لقانون بير – لامبرت عند مدى 10 - 0.2 مايكروغرام مل$^{-1}$ لكلا الطريقتين . معاملات الارتباط وحدود الكشف كانت0.999 ، 0.37 و 0.998، 0.426 للطريقة(ا) و(ب) على التوالي . طبقت الطرق الحالية بنجاح لتقدير السيليكوكسب كميا بالمستحضرات الصيدلانية بدقة جيدة .