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# Extractive Spectrophotometric Methods for the Determination of Celecoxib

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**Abstract:** Two simple, rapid and sensitive spectrophotometric methods for the determination of celecoxib in pure form and in pharmaceutical preparations have been developed. These methods are based on the formation of ion-pair complexes between the drug and indicators (BCG and BPB). These reactions give colored products having maximum absorbance at 428 and 436 nm for BCG and BPB, respectively. Beer's law is obeyed in the concentration ranges 5- 30 μgmL$^{-1}$ and 2-20 μgmL$^{-1}$ for celecoxib using BCG and BPB reagents. The molar absorptivities are 7.627×10$^{3}$ and 7.726×10$^{3}$ L mol$^{-1}$ cm$^{-1}$ and the Sandell (*S*) sensitivities are 0.050 μg cm$^{-2}$ 0.049 and μg cm$^{-2}$ using BCG and BPB reagents, respectively, which indicate the high sensitivity of the proposed method.
**Keywords :** Celecoxib; Extractive spectrophotometry; Ion-pair complex; BCG; BPB.

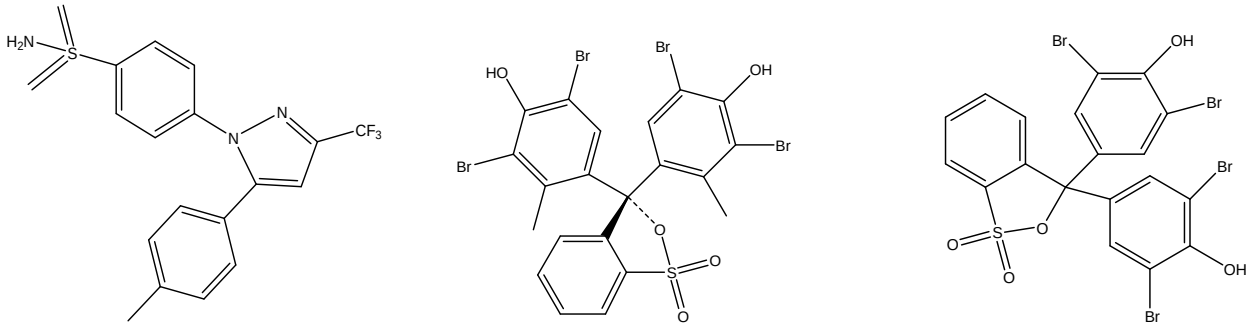
## 1. Introduction

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-l] bezensulfonamide, 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 to reduce numbers of colon and rectum polyps in patients with familial adenomatous polyposis[1,2]. It is known under the brand name Celebrex or Celebra for arthritis and Onsenal for polyps. Celecoxib is available by prescription in capsule form.

The mechanism of action of celecoxib is believed to be due to inhibition of prostaglandin synthesis. Unlike most NSAIDs, which inhibit both types of cyclooxygenases (COX-1 and COX-2), celecoxib is a selective noncompetitive inhibitor of cyclooxygenase-2 (COX-2) enzyme. It binds with its polar sulfonamide side chain to a hydrophilic side pocket region close to the active COX-2 binding site. Both COX-1 and COX-2 catalyze the conversion of arachidonic acid to prostaglandin (PG) H2, the precursor of PGs and thromboxane[3].

There are some HPLC methods for the determination of Celecoxib in pharmaceutical preparations[4-7]. It was also reported using HPTLC[8]. In addition some UV spectrophotometric methods have been developed for determination of celecoxib[9-11]. Only one visible spectrophotometric method was reported based on the reaction with o-phenanthroline and ferric chloride[12]. Extractive spectrophotometric methods have been widely used for determination of pharmaceutical compounds. A rapid extractive spectrophotometric method has been developed based on the formation of a chloroform soluble ion-pair complex between carvedilol and bromocresol green in an acidic medium[13]. Moreover there are other extractive methods based on ion-pair complexation for determination of drugs in pure and dosage forms[14-17]. In this work we report two simple visible spectrophotometric methods for determination of celecoxib in pure and pharmaceutical preparations which are

based on the reaction between drug and BCG (method A) and BPB (method B) which leads to the formation of ion-pair complexes.




(a) (b) (c)

**Scheme 1. Structure of a) celecoxib b)BCG and c)BPB**

## 2. Methods

### 2.1. Instrumentation

A Ray Leigh UV-1800 spectrophotometer was used for all absorbance measurements and pH-meter model

metrohom 825 was used to measure the pH.

### 2.2. Chemicals and reagents

All chemicals used were of analytical grade. Double distilled water was used for preparation of reagent solutions. Bromocresol green (BCG) and bromophenol blue (BPB) were provided from Merck company and used without further purification. BCG and BPB solutions ($1.0 \times 10^{-4}$ M) were prepared in methanol and then diluted with water. Disodium hydrogen phosphate-hydrochloric acid buffer solution was prepared in pH=3. Aria company (Tehran- Iran) supplied pure powder of celecoxib as a gift. The commercially available tablets of celecoxib contain 100 mg were used.

### 2.3. Standard solutions

Stock solution of celecoxib (1 mg $mL^{-1}$) was prepared by dissolving of 100 mg of celecoxib in 100 mL methanol. The working standard solution (100 µg $mL^{-1}$) was prepared by stepwise dilutions of the stock solution with methanol.

### 2.4. Procedures

#### 2.4.1. Construction of calibration curve

Aliquots containing from 5-30 µg of celecoxib for method A and 2-20 µg of celecoxib for method B were transferred into a series of calibrated flasks and 2 mL of dye solutions (BCG or BPB) and 3 mL of buffer solution (pH=3) were added to each flask and made up with HCL (0.1 N). For extraction step, the solutions were transferred into the separating funnels. The funnels were shaken vigorously for 3 minutes with chloroform (5 × 2 mL) then allowed to stand for clear separation of the two phases. After separation of the phases the organic layer should be filtered and the absorbance of the organic phase was measured at 428 and 436 nm for BCG and BPB respectively, against a reagent blank similarly prepared.

### 2.4.2. Procedure for dosage forms

Twenty tablets were accurately weighed and powdered. The tablet powder equivalent to 100 mg of celecoxib was dissolved in 100 mL methanol. This solution was suitably diluted with methanol for preparing working solutions and then preceded as described above for pure drug.

## 3. Results and discussion

### 3.1. Spectral characteristics

These methods are based on the formation of colored ion-association complexes with bromocresol green and bromophenol blue in pH=3 which were extracted in chloroform. The absorption spectra of complexes with BCG and BPB under the optimized conditions have been shown in Fig. 1 (a) and (b). The absorption bands of the complexes are located at 428 and 436 nm for BCG and BPB, respectively.

Celecoxib is a nitrogenous drug which is present in positively charged protonated form and anionic dyes such as BCG and BPB are mainly in anionic forms. The proposed method is based on the formation of a salt (ion-pair) which contains an amine in its ionized form (celecoxib) and an ionized dye and then was extracted into an organic solvent such as chloroform.

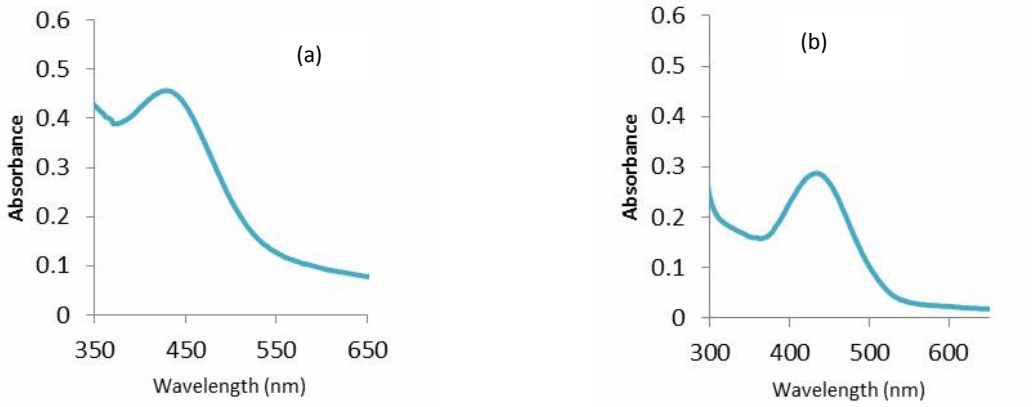



**Figure 1. (a) Absorption spectra of celecoxib-BCG complex. (b) Absorption spectra of celecoxib-BPB complex**

### 3.2. Method optimization

The optimizations of reaction conditions were studied carefully to achieve the highest sensitivity and most stability for the colored complex. The effects of some parameters on the extraction of the ion-pair complexes were investigated such as pH and time and the optimized conditions have been selected for further experiments.

### 3.2.1. Effect of pH

Effect of pH was investigated for both methods over the pH range 2-5 using disodium hydrogen phosphate-hydrochloride acid buffer solutions with different pH. It was found that pH=3 is an optimum pH for both methods and the extracted complexes have maximum absorbance in this pH (Fig. 2 a and b).

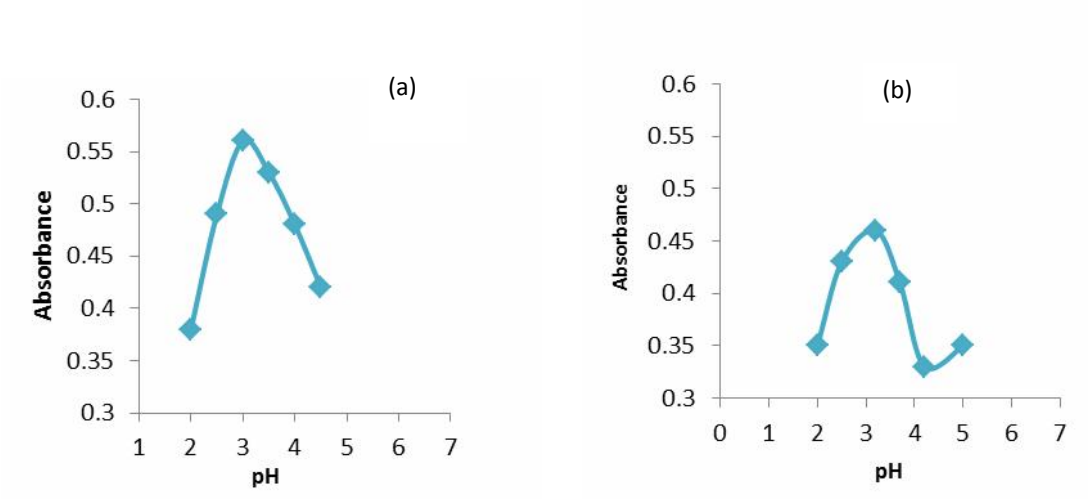



**Figure 2. (a) Effect of pH on formation of celecoxib-BCG complex. (b) Effect of pH on formation of celecoxib – BPB complex**

### 3.2.2. Effect of reagent concentrations

When we followed the general procedure it is found that 2 ml of $10^{-4}$ M reagent solution, gives maximum and constant absorbance for both methods. The results are shown in Fig 3 (a) and (b).

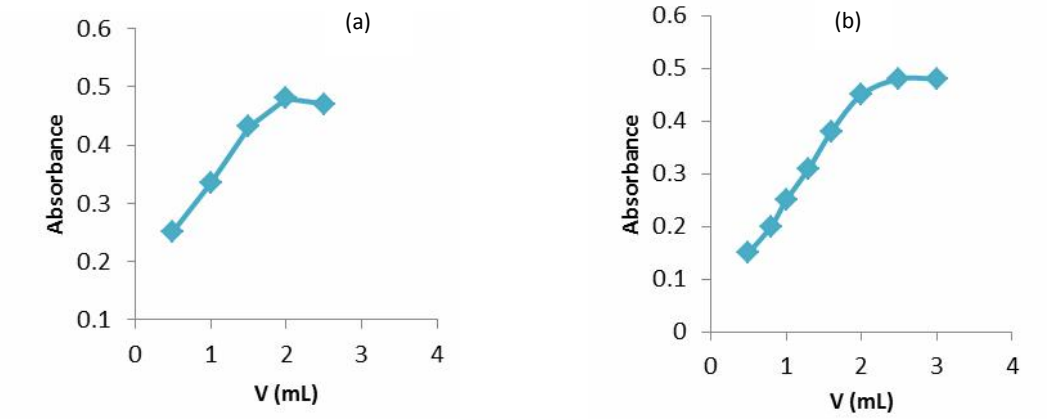



**Figure 3. (a) Effect of reagent concentrations for BCG. (b) Effect of reagent concentrations for BPB method (drug solution 20 µg $mL^{-1}$ and dye solutions $10^{-4}$ M)**

### 3.2.3. Effect of time

Effect of time was investigated in this work and it was found that complete color development was attained after 10 min for BCG and 20 min for BPB. The effect of time has been shown in Fig. 4 (a) and (b).

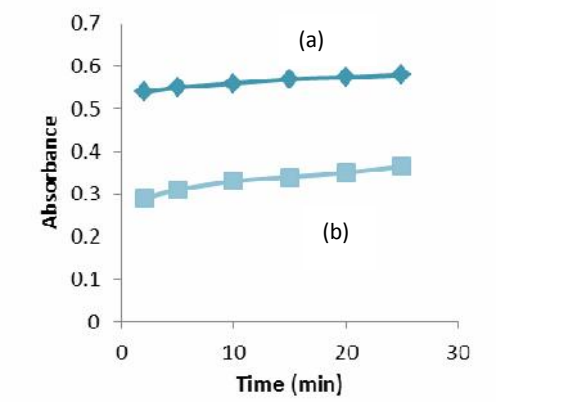
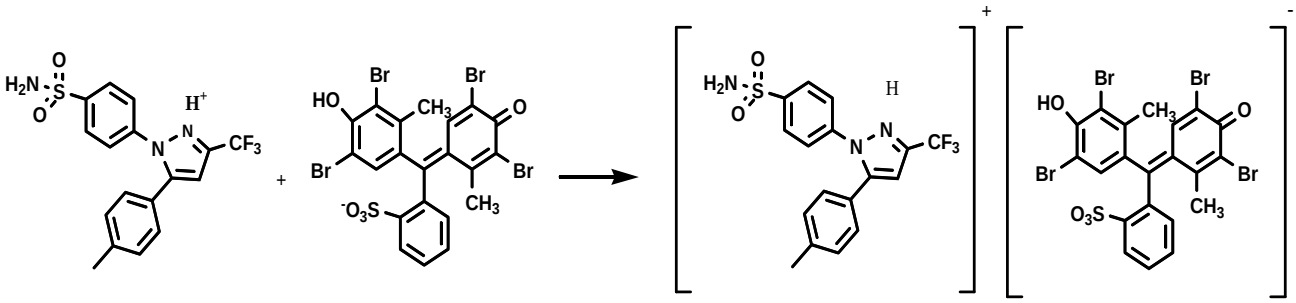



**Figure 4. (a) Effect of time for BCG method. (b) Effect of time for BPB method (drug solution 20 µg mL$^{-1}$ and dye solutions 10$^{-4}$ M )**

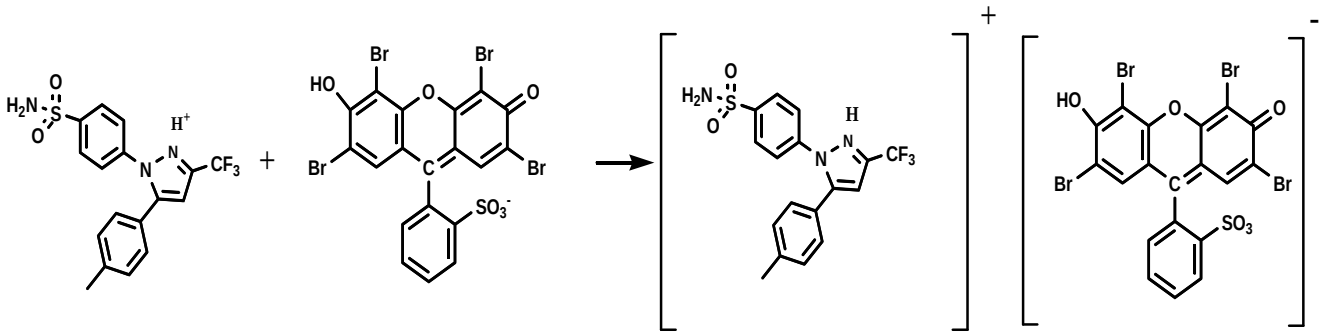
### 3.3. Mechanism of reaction

#### 3.3.1. Proposed mechanism of formation of ion-pair complexes

Ion-association is a chemical reaction whereby ions of opposite electrical charge come together in solution to form a distinct chemical entity. In this work an ion- pair complex is formed between an amine in its ionized form that has a positive charge in the selected pH and an ionized dye (BCG or BPB) that has a negative charge. The proposed structure of ion–pair complexes is shown in Scheme 2 (a) and (b).




**Scheme 2. (a) Proposed structure of celecoxib-BPB complex**




**Scheme 2 (b) Proposed structure of celecoxib-BCG complex**

### 3.3.2. Stoichiometry of the ion-pair complexes

Molar ratio method is applied in order to determine the suitable ratio between celecoxib and BCG or BPB reagents. The results demonstrate that 1:1 complexes were formed between the drug and BCG and BPB reagents. The results have been shown in Fig. 5 (a) and (b).

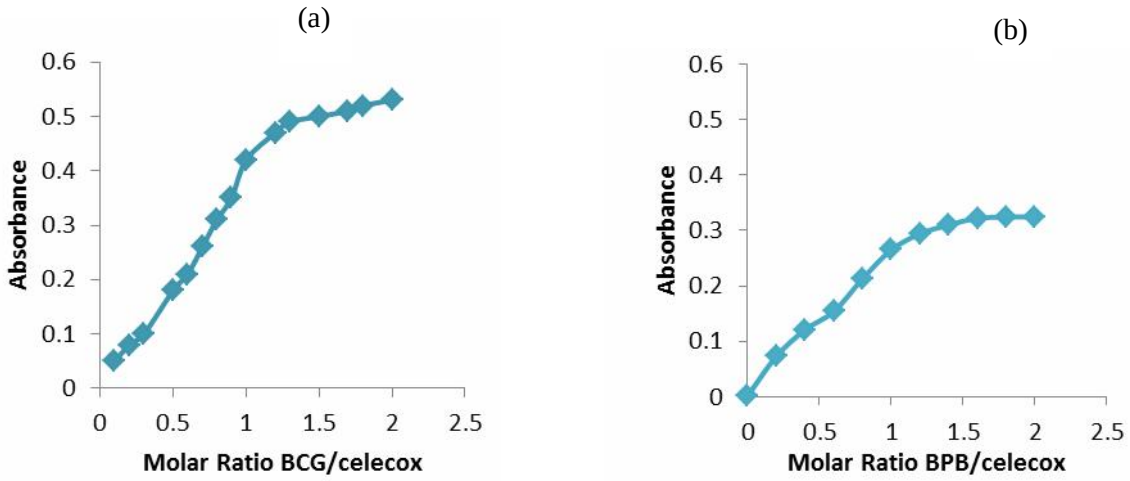



**Figure 5. (a) Molar ratio for BCG method. (b) Molar ratio for BPB**

### 3.4. Method validation

### 3.4.1. Linearity and range

General procedure was followed in optimum conditions such as time, reagent concentrations and then the calibration curves can be constructed by plotting absorbances versus concentrations (Fig. 6 a and b). Beer' s law is obeyed over the cocentration ranges of 5-30 µg $ml^{-1}$ for BCG and 2-20 µg $ml^{-1}$ for BPB method. Table 1 shows the different analytical parameters obtained for both method. In addition determination of celecoxib in pure form hase been investigated and ther resulte have been shown in Table 2.

**Table 1. Analytical parameters of proposed**

| Parameter | BCG | BPB |
|---|---|---|
| $\lambda_{max}$ (nm) | 428 | 436 |
| Beer's law limits(µg/ml) | 5-30 | 2-20 |
| ε(1mol$^{-1}$ cm$^{-1}$) | 7.627 × 10$^3$ | 7.726 × 10$^3$ |
| Regression equation | Y=0.0115X +0.0984 | Y=0.018X +0.0187 |
| Correlation coefficient (r) | 0.996 | 0.9952 |
| Relative standard deviation(%RSD) | 0.909 | 0.636 |
| Limit of detection (µg ml$^{-1}$) | 0.247 | 0.167 |
| Limit of quantification (µg ml$^{-1}$) | 0.824 | 0.556 |
| Sandell sensitivity(µg cm$^{-2}$) | 0.050 | 0.049 |

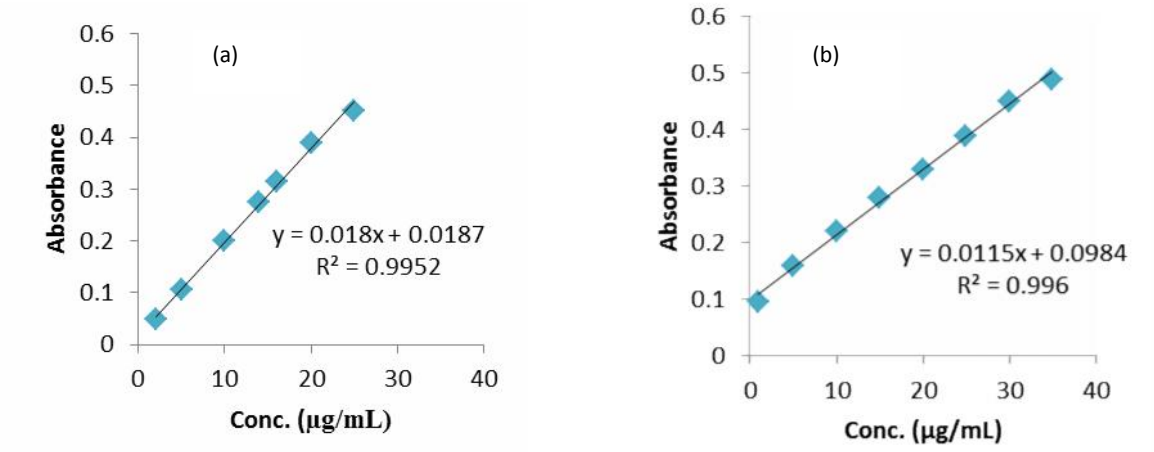



**Figure 6. (a) Calibration curve for BCG method. (b) Calibration curve for BPB method**

**Table 2. Spectrophotometric determination of celecoxib in**

| Proposed method | taken (µg $mL^{-1}$) | found ( µg $mL^{-1}$) | Recovery (%) | RSD(%) |
|---|---|---|---|---|
| BCG | 15.00 | 14.91 | 99.40 | 1.14 |
| | 25.00 | 25.09 | 100.36 | 1.07 |
| BPB | 10.00 | 9.98 | 99.88 | 0.76 |
| | 15.00 | 14.89 | 99.27 | 0.727 |

### 3.5. Application of the proposed method

The proposed methods were successfully applied to the determination of celecoxib in tablets. Table 3 shows the results of standard addition method for determination of celecoxib.

Table 3 also shows that recovery percentages are close to 100% too and ranged from 99.06 to 100.24 that indicate the high efficiency of the methods.

**Table 3. Spectrophotometric determination of celecoxib in pharmaceutical preparations by standard addition method**

| preparations | BCG method | | | | BPB method | | | |
|---|---|---|---|---|---|---|---|---|
| | Taken (µg $mL^{-1}$) | Added (µg $mL^{-1}$) | Found (µg $mL^{-1}$) | Recovery (%) | Taken (µg $mL^{-1}$) | Added (µg $mL^{-1}$) | Found (µg $mL^{-1}$) | Recovery (%) |
| Celecoxib | 5.00 | 10.00 | 14.86 | 99.06 | 5.00 | 10.00 | 15.018 | 100.12 |
| | 10.00 | 10.00 | 19.86 | 99.32 | 10.00 | 10.00 | 19.97 | 99.85 |
| Cebex | 5.00 | 10.00 | 15.03 | 100.17 | 5.00 | 10.00 | 15.01 | 100.06 |
| | 10.00 | 10.00 | 20.05 | 100.24 | 10.00 | 10.00 | 20.01 | 100.05 |

**Table 4. Comparison between accuracy of proposed methods and reference**

| | proposed method (BCG) | | | proposed method (BPB) | | | Reference method | | |
|---|---|---|---|---|---|---|---|---|---|
| Labled (mg) | found ( mg ) | Recovery (%) ± SD | SE | found ( mg ) | Recovery (%) ± SD | SE | found (mg) | Recovery (%)± SD | SE |
| 100 | 100.13 | 100.13±0.83 | 0.13 | 98.64 | 98.64 ± 0.75 | 1.2 | 100.13 | 100.13±0.5929 | 0.2651 |

In Table 4 we compare the recovery percentage of the present method with the reference method which is a simple UV spectrophotometric method for determination of celecoxib. The determinations were carried out at maximum absorption band 255 nm in chloroform solvent[18]. The results show that the proposed method is precise and accurate.

## 4. Conclusions

The data given above reveal that the proposed methods are simple, rapid, accurate, precise and economical for the routine analysis of celecoxib in pharmaceutical quality control laboratories. With these methods, one can do the analysis with pace at low cost without losing accuracy. The proposed methods have been successfully applied to the determination of celecoxib in tablet forms as well.

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## References

1. Pietrzyle J, Clyde FW, 1979. The pharmacological basis. New York, McGraw-Hill. 42: 25-31.
2. Boyce EG, Breen GA, 1999. Clinical pharmacokinetics of antagonists. 34: 405-417.
3. Penning TD, 1997. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxy genase-2 inhibitors. Pharm Biomed Anal, 40: 1347-1365.
4. Baboota S, Faiyaz S, Ahuja A, Ali J, Shafiq S, Ahmad S, 2007. Devalopment and validation of a stability-indicating HPLC method for analysis celecoxib. Acta Chromatographica, 12:451-456.
6. Starek M, 2012. Review of the applications of different analytical techniques for coxibs research. Talanta, 85: 8-27.
7. Hashem H, Toundelberg C, Jira T, 2010 Simple UV Spectrophotometric Determination of Celecoxib in Pure Form and in Pharmaceutical Formulations. Anal, 71: 91-98.
8. Sante R T, Swati P, Sachin K J, 2004. HPLC method for Determination of Celecoxib. Planar Chromatogr, 17: 61-65.
9. Saha RN, Sajeev C, Jadhav PR, Patil SP, Srinivasan NJ, 2002. UV Spectrophotometric Determination of Celecoxib. Pharm Biomed Anal, 28: 741-750.
10. Sahu SK, Azam MA, Dash DK, Banarjee M, 2009. UV Determination of Celecoxib. Indian Chemistry. 86: 308-311.
11. Sankar, DG, 2001. Studies on the Development of Celecoxib Transdermal Patches. Indian Pharm. 63: 521-523.
12. Pillai S, Singhvi I, 2006. Spectrophotometric Determination of Celecoxib with with o-phenanthroline. Asian Chemistry, 18: 20-25.
13. Verma JK, Syed HA, 2007. Extractive spectrophotometric method for determination of carvedilol in tablets . Indian Pharm Sci, 69:303-304.
14. Souri E, Kaboodari, Adib N, Amanlou M, 2013. A New extractive spectrophotometric method for determination of rizatriptan dosage forms using bromocresol green. . Daru Pharml Sci, 21: 2008-223.
15. Rania A, Hassan S, Magda Y. Mammli EL, Abdalli A, Shalabi A, 2012. New Extractive Spectrophotometric Method for the Determination of Gatifloxacin and Cefotaxime Sodium in Pure and Pharmaceutical Dosage Forms.. Oriental Chem. 28: 639-650.
16. Shah J, Rasul M Manzoor S, 2008. Extractive Spectrophotometric Methods for Determination of Clarithromycin in Pharmaceutical Formulations Using Bromothymol Blue and Cresol Red. Chinese Chem Soci, 55:1107-1112.

17. Lotfy Saber A, Owes G. Extractive Spectrophotometric Determination of Anti-Inflammatory Drug Nimesulide in Pharmaceutical Formulations and Human Plasma. Food Drug Anal, 19: 429-436.
18. Revathi R, Venkatesha Perumal S. Sudharshini A, AnsarM, Dinesh A, 2011. Simple UV Spectrophotometric Determination of Celecoxib in Pure Form and in Pharmaceutical Formulations. Pharm Science, 65: 49-52.

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