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# DEVELOPMENT AND VALIDATION OF FT-IR SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF DICLOFENAC SODIUM AND RABEPRAZOLE SODIUM IN TABLET DOSAGE FORM

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**ABSTRACT**

For the purpose of performing simultaneous determinations of diclofenac sodium and rabeprazole sodium in tablet dosage form, an easy-to-use, cost-effective, accurate, precise and validated diffuse reflectance infrared fourier transform spectroscopy method has been developed. Measurement of the spectral wave number of the infrared band corresponding to the $NH_2$ group stretch at 3388 $cm^{-1}$ for diclofenac sodium and the S=O group at 1274 $cm^{-1}$ for rabeprazole sodium is included in the newly developed approach. The approach was found to be linear over the range of 1%-6% weight by weight for diclofenac sodium and 0.2%-1.2% weight by weight for rabeprazole sodium, and demonstrated a correlation coefficient ($r^2$) of 0.991 and 0.991, respectively. The method that was developed was validated to ensure that it met the requirements set forth by the ICH regarding linearity, precision, accuracy, limit of detection, and limit of quantitation. In addition to this, the behaviours of deterioration of the solid state of diclofenac sodium and rabeprazole sodium were investigated by exposing them to photolysis, sunlight and thermal degradation.

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## Introduction

Chemically, diclofenac sodium is known as Sodium-2-[2-(2,6 dichloroanilino)phenyl]acetate. In addition to treating osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. It is also used to treat a wide range of other inflammatory conditions that are not related to rheumatoid arthritis (1-3). Thechemicalnameforrabeprazoleis 2-([4-(3-Methoxypropoxy)-3-methyl-2 pyridyl]methylsulfinyl)-1H-benzimidazole sodium. It is classified as an example of an inhibitor of the proton pump. Atthesecretorysurfaceofthestomachparietalcell,itdoesthisviablockingtheH+/K+-ATPaseenzymesystem.Thisbringsinareductioninthequantityofgastricacidthatissecretedasaconsequence.

Clinically, rabeprazole is administered to patients suffering from acid-peptic diseases such esophageal, gastric and duodenal ulcers in order to promote healing, alleviate symptoms and avoid relapse [4].

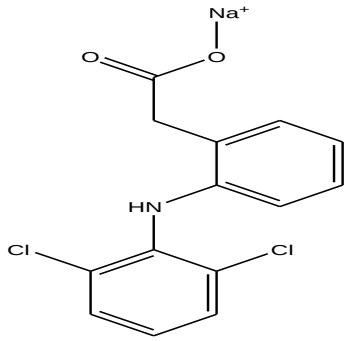


**Figure1: Structure of Diclofenac sodium**

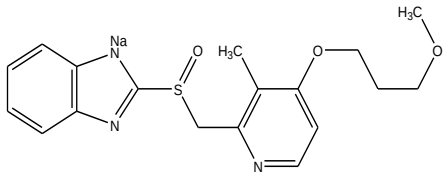


**Figure2: Structure of Rabeprazole sodium**

A review of the relevant literature revealed that, various reports of individual estimation of rabeprazole from its formulations including, the stability of rabeprazole in aqueous media [5], analytical methods including HPLC [6], UV [7], determination of the enantiomeric configuration [8] and photodegradation products estimation by UV and HPLC [9,10] have been reported. A study of the relevant literature indicated that there are few reports on analytical methods for estimating individual drug diclofenac from formulations using methods such as potentiometric and fluorimetric determination [11]. In spite of this, there were no methods that had been published up until this point for the simultaneous determination of both drugs through the use of FT-IR spectroscopy as a tool for simultaneous estimation. The development of a novel FT-IR method for the routine analysis of diclofenac sodium and rabeprazole sodium in combined tablet dosage form is the aim of the research work.

## Materials and methods

### Apparatus and instruments:

The FT-IR spectrophotometer known as the IR-Affinity-1, which was made in Japan by Shimadzu Corp., was the instrument that was utilised for the objectives of data collecting and analysis. This instrument had a diffuse reflectance sampling interface built into it, and it was connected to a computer that had Shimadzu IR solution software running on it. In addition to this, it makes use of a high-energy, long-life ceramic light source and is fitted with a DLATGS detector. The FT-IR spectra were acquired throughout the entire range of 400-4000 cm-1 using 45 scans and a resolution of 8 cm-1 during the entire process. Analytical weighing balance: A named, model AA-2200. [Max. 200 g, Min. 0.01 g; e = 0.0001 g].

**Chemical and reagent:**

Wockhardt Pvt. Ltd. in Aurangabad was kind enough to provide us with working standard drug sample as gift sample. R-Clonac, a commercially available formulation consisting of diclofenac sodium 100 mg and rabeprazole sodium 20 mg, was acquired from local market. As the working diluent, pure KBr of analytical grade was utilised and throughout the process, only calibrated glassware was utilised.

**Preparation of working standard:**

Pure drugs weighing 10 milligrammes of diclofenac sodium and 2 milligrammes of rabeprazole sodium accurately were combined with 990 milligrammes and 998 milligrammes of KBr (of spectrometric grade) and thoroughly triturated in order to produce a homogenous combination.

**Selection of analytical wave number:**

Both drugs' working standard of concentrations 1% and 0.2% w/w were scanned in the infrared range of 4000-400 $cm^{-1}$, with a resolution of 4 scans and 45 scans respectively. The wave number was chosen accordingly, in order to eliminate the possibility of interference caused by one drug with another, so that one functional group of one drug should not be present in another drug. The NH2 functional group was chosen for the DCL, and the wave number that was determined was 3388 $cm^{-1}$. The S=O functional group was chosen for RAB, and the wave number that was discovered was 1274 $cm^{-1}$.

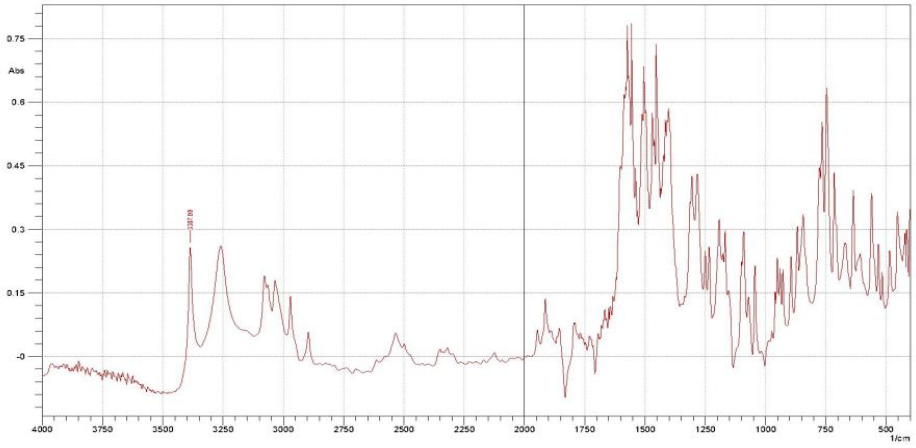


**Figure3: FT-IR Spectrum of DCL**

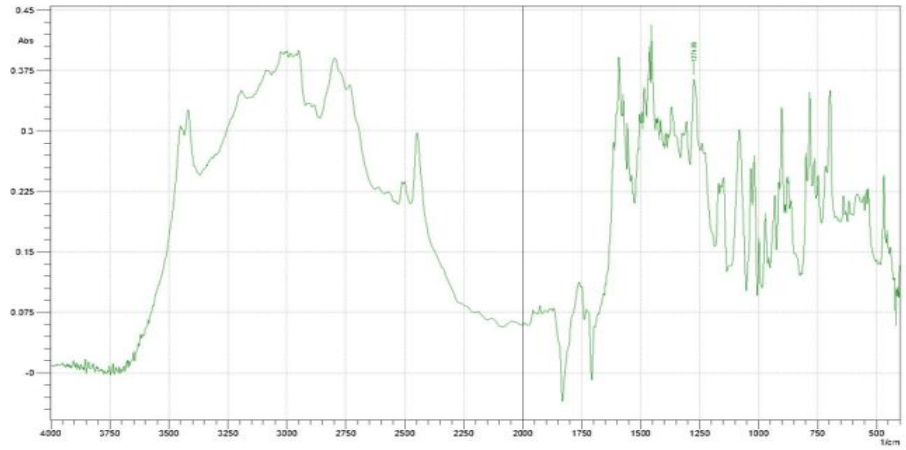


**Figure4: FT-IR spectrum of RAB**

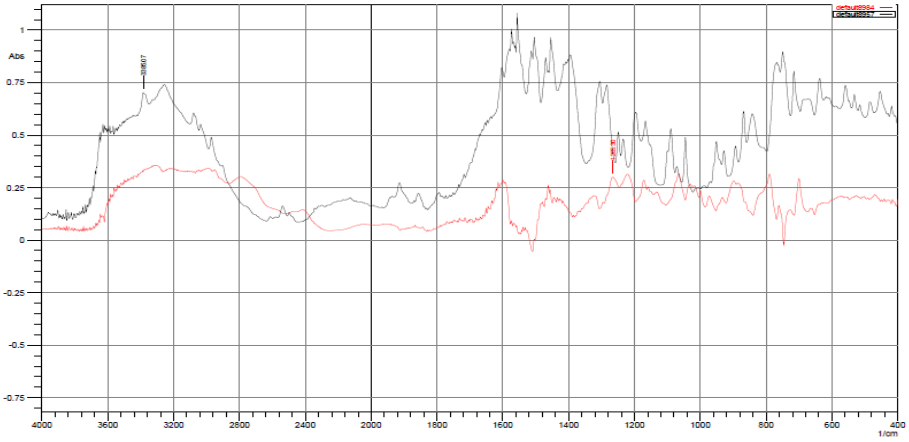

**Figure 5: FT-IR spectrum of overlain spectra of standard DCL and RAB**

**Selection of analytical concentration range and linearity study:**

In order to achieve concentrations ranging from 1-6% w/w and 0.2-1.2% w/w for DCL and RAB, respectively, pure drug samples of both RAB and DCL were diluted with KBr. Using KBr as a blank, we were able to determine that the peak intensity of these dilutions fell somewhere between 3388 $cm^{-1}$ for DCL and 1274 $cm^{-1}$ for RAB. It was found that a linear relationship existed when peak intensity was plotted versus concentration was plotted.

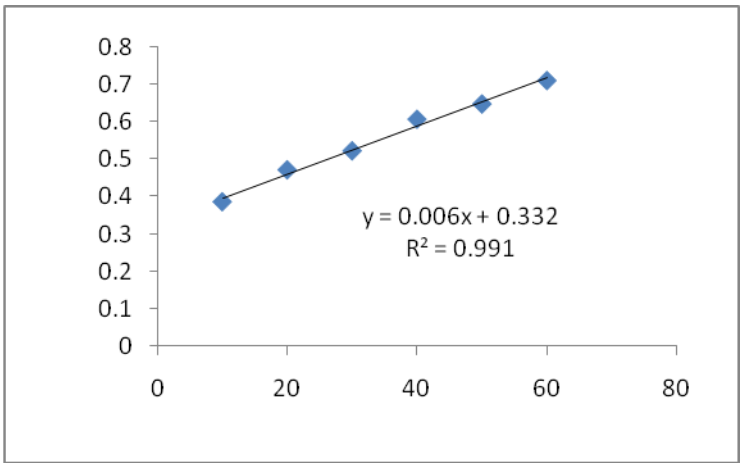


**Figure 6: Calibration curve of DCL**

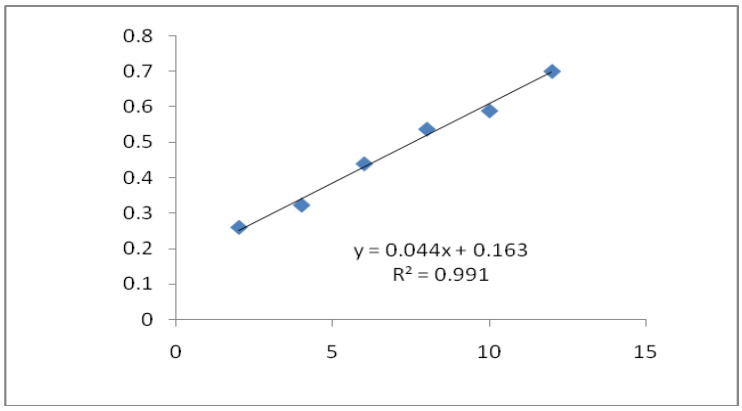


**Figure7: Calibration curve of RAB**

**Analysis of marketed tablet formulation:**

Twenty tablets of the formulation that is available commercially were measured to a high degree of accuracy, and the average weight of those tablets was computed. After that, these tablets were ground into a fine powder, and then out of that powder, a quantity was taken that weighed equivalent to 10 mg of DCL and 2 mg of RAB. After combining it with 988 mg of KBr, the resulting dilution for DCL was found to be 1% w/w, whereas the dilution found for RAB was 0.2% w/w.

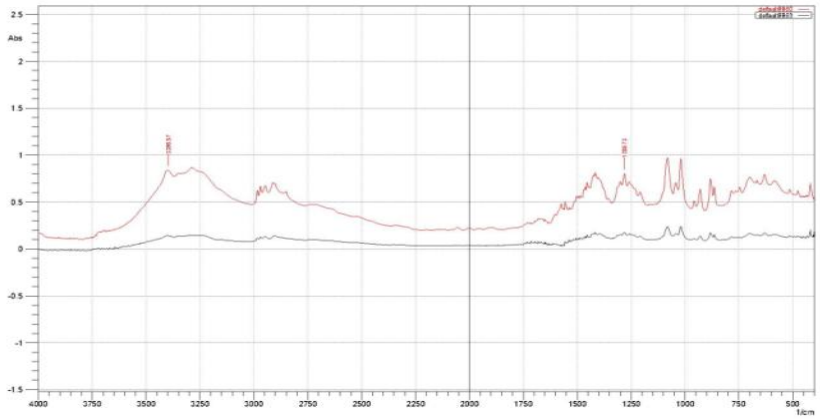

**Figure 8: FT-IR spectra of Tablet excipients and formulation**

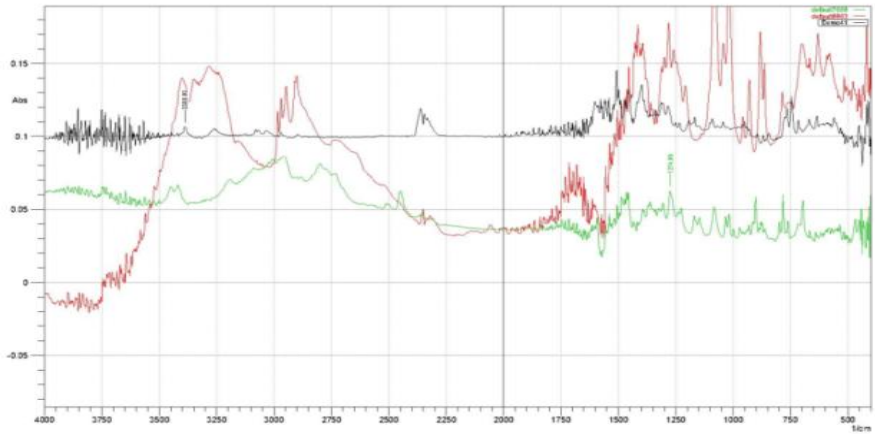

**Figure 9: FT-IR overlain of tablet excipients and standard drug of DCL and RAB**

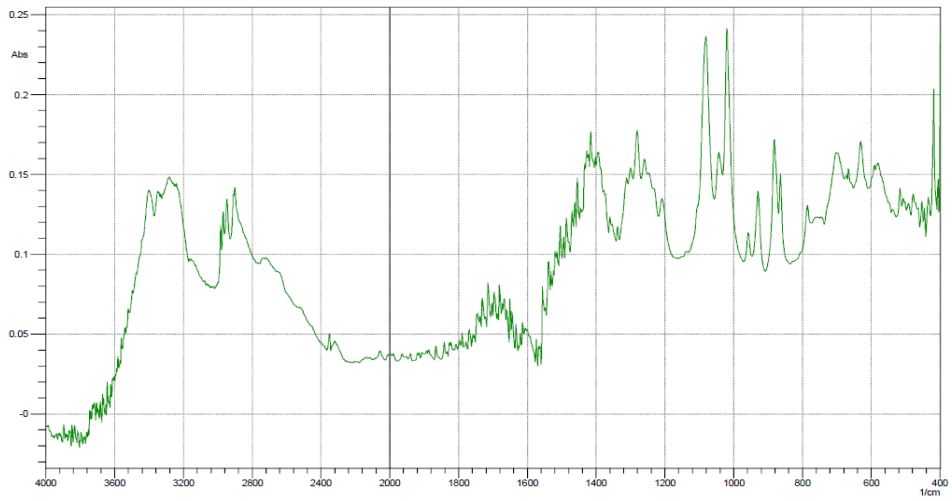


**Figure 10: FT-IR overlain of tablet excipients**

**Method Validation**

**Linearity:**

In order to conduct the linearity investigation, standard dilutions of 1, 2, 3, 4, 5 and 6% w/w were prepared for DCL, and 0.2, 0.4, 0.6, 0.8, 1 and 1.2% w/w were prepared for RAB. After doing so, for each concentration the calibration graph was plotted, as the concentration versus the intensity of the DCL and RAB, respectively. The linearity of the suggested technique was determined to be in between 1-6% w/w for DCL and between 0.2-1.2%w/wforRAB.

**Precision:**

Inter-day and intra-day variation studies were conducted in order to evaluate the method's level of precision. When conducting intraday investigations, working dilutions of the sample were evaluated in triplicate within a single day and the percentage relative standard deviation (% RSD) was computed.

For the purpose of carrying out the calculation, Formula 1 was utilised.

$$\%\,Estimation = \frac{IntensityofSample}{IntensityofStandard} X \frac{Wt.ofstd.}{wt.ofsample} X\ 100 \text{ -------- (1)}$$

**Accuracy:**

In accordance with the guidelines provided by the ICH, the recovery studies were conducted at three distinct levels: 80%, 100%, and 120%. This was done so that the accuracy of the proposed methods could be determined.

The tablet is said to have 100 mg of DCL and 20 mg of RAB, as per label claim. When conducting recovery studies, various levels of the standard concentration, based on 80%, 100%, and 120%, are created, and the % mean recoveries are determined. The material was carefully combined before being examined. The percentage of recovery was determined by applying the formulas 2 and 3.

$$Amountfound = \frac{TotalamountaddedXAmountestimated}{100} \text{ --------- (2)}$$

$$\%\,recovery = \frac{LabelClaimXAmountfound}{Totalamountadded} \text{ ---------- (3)}$$

**LOD AND LOQ:**

The ICH guideline outlines multiple methodologies that can be utilised to ascertain the detection and quantitation thresholds. A visual inspection, a signal-to-noise ratio calculation, the application of the response's

standard deviation and the calibration curve's slope are some of the methods. In the present investigation, the LOD and LOQ were determined utilising the third approach i.e. by application of standard deviations and were computed with the aid of the equations given below:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, which is used as a measure of noise, and S is the corresponding calibration curve's slope. Standardpeakintensityandpercentage of degradationandpercentage of assaywascalculated.

**Result and Discussion**

The absorbance intensity of 3388 $cm^{-1}$ and 1274 $cm^{-1}$ was found in the FTIR spectra for pure samples of DCL and RAB, respectively. Because the dilution in dry potassium bromide did not have a significant impact on the intensity of absorbance bands that resulted from DCL and RAB. Hence, we decided to make use of dry potassium bromide as a diluent for this particular study. Themostprominentabsorbanceband,whichcorrespondstotheNH2group,wascentredintheintensityof3388cm-1for diluted samples of DCL in dry potassium bromide, and the S=Ogroup,which was centred in the intensity of 1274 cm-1 for diluted samples of RAB in dry potassium bromide,was with in 2.0 absorbance unit. As can be seen in figure 7, the intensities of 3388 $cm^{-1}$ and 1274 $cm^{-1}$ were employed in the creation of a calibration curve for DCL and RAB, respectively. In the case of DCL, the calibration curve can be described by the equation y = 0.006x+0.332. In the case of RAB, the calibration curve can be summarised by the equation y=0.044x+0.163. Initially, different ranges of samples were tried. We were able to establish a calibration curve that had good linearity and had arrange that went from 1-6% w/w for DCL and 0.2-1.2% w/w for RAB in KBr. For both the drugs, DCL and RAB, the correlation coefficient for the calibration curve was 0.991. The linear regression equation that corresponds to DCL was y = 0.006xs+0.332 and the linear regression equation that corresponded to RAB was y = 0.044xs+0.163.

**Table 1: Linearity study data of DCL**

| Sr.no. | Concentration (% w/w) | Intensity (3388$cm^{-1}$) |
|---|---|---|
| 1 | 1 | 0.385 |
| 2 | 2 | 0.471 |
| 3 | 3 | 0.522 |
| 4 | 4 | 0.607 |
| 5 | 5 | 0.648 |
| 6 | 6 | 0.711 |

**Table 2: Linearity study data of RAB**

| Sr.no. | Concentration (%w/w) | Intensity ($1274cm^{-1}$) |
|---|---|---|
| 1 | 0.2 | 0.259 |
| 2 | 0.4 | 0.322 |
| 3 | 0.6 | 0.439 |
| 4 | 0.8 | 0.537 |
| 5 | 1 | 0.589 |
| 6 | 1.2 | 0.701 |

**Table3: Analysis of tablet formulation**

| Sr. no. | Label claim mg/tab | | Amount found (mg/tab) | | %assay | |
|---|---|---|---|---|---|---|
| | DCL | RAB | DCL | RAB | DCL | RAB |
| 1 | 10 | 2 | 9.8 | 1.96 | 98.03 | 98.49 |
| 2 | 10 | 2 | 10.06 | 2.05 | 100.65 | 102.5 |
| 3 | 10 | 2 | 10.21 | 2.06 | 102.17 | 103.17 |
| 4 | 10 | 2 | 9.97 | 1.97 | 99.78 | 98.99 |
| 5 | 10 | 2 | 10.28 | 2.07 | 102.83 | 103.51 |
| 6 | 10 | 2 | 10.04 | 2.01 | 100.43 | 100.66 |

**Table 4: Statistical validation: analysis of tablet formulation**

| Name of the drug | Mean | SD | %RSD |
|---|---|---|---|
| DCL | 100.64 | 1.71 | 1.69 |
| RAB | 101.22 | 2.16 | 2.13 |

*Indicates average of six determinations

**Table 5: Linear regression data for calibration curve of DCL and RAB**

| Nameof the drug | Linearity range(%w/w) | $r^2$ | Slope | Intercept |
|---|---|---|---|---|
| DCL | 10-60 | 0.991 | 0.006 | 0.332 |
| RAB | 2-12 | 0.991 | 0.044 | 0.163 |

The coefficient of variation (%RSD) was used to express the precision, while the mean and standard deviation were utilised to express the accuracy. The %RSD value for three DCL samples was observed to be 0.901% during intra-day precision studies, it was observed to be 1.066% during inter-day precision studies. For RAB, the %RSD value for three DCL samples was observed to be 0.597% for intra-day precision and 0.41% for inter-day precision studies. Both the intraday and the interday precision results were within the variable limitations that were considered acceptable.

**Table 6: Repeatability data**

| Sr.no. | Concentration(mg) | | Absorbance | | % Recovery | |
|---|---|---|---|---|---|---|
| | DCL | RAB | DCL | RAB | DCL | RAB |
| 1 | 10 | 2 | 0.458 | 0.595 | 99.78 | 99.49 |
| 2 | 10 | 2 | 0.460 | 0.598 | 100.21 | 100 |
| 3 | 10 | 2 | 0.459 | 0.599 | 100 | 100.16 |
| 4 | 10 | 2 | 0.450 | 0.589 | 98.03 | 98.49 |
| 5 | 10 | 2 | 0.472 | 0.619 | 102.83 | 103 |
| 6 | 10 | 2 | 0.461 | 0.602 | 100.43 | 100.6 |

**Table7: Statistical validation of repeatability data**

| Name of the drug | Mean | SD | %RSD |
|---|---|---|---|
| **DCL** | 100.21 | 1.54 | 1.53 |
| **RAB** | 100.29 | 1.51 | 1.50 |

*Indicates average of six determinations

**Table 8: Precision data of marketed formulation**

| Sr.no. | Interval of time | Concentration(mg) | | %recovery | |
|---|---|---|---|---|---|
| | | DCL | RAB | DCL | RAB |
| 1 | 0 hr | 10 | 2 | 100.21 | 100.50 |
| 2 | 3 hr | 10 | 2 | 101.96 | 100.16 |
| 3 | 6 hr | 10 | 2 | 100.65 | 101.33 |
| 4 | Day-1 | 10 | 2 | 100.65 | 100.5 |
| 5 | Day-2 | 10 | 2 | 98.69 | 100.16 |
| 6 | Day-3 | 10 | 2 | 100.4 | 101 |

**Table 9: Statistical validation of intra-day precision data**

| Name of the drug | Mean | SD | %RSD |
|---|---|---|---|
| **DCL** | 100.94 | 0.91 | 0.901 |
| **RAB** | 100.66 | 0.601 | 0.597 |

**Indicates average of three determinations*

**Table10: statistical validation of inter-day precision data**

| Name of the drug | Mean | SD | %RSD |
|---|---|---|---|
| **DCL** | 99.91 | 1.066 | 1.066 |
| **RAB** | 100.55 | 0.422 | 0.41 |

**Indicates average of three determinations*

Standard addition method was used to recover pure drug at three different levels (80%, 100%, and 120% w/w of label claim), and the assay method's accuracy was determined based on the results of this evaluation, which can be found in tables 11 and 12.

At various concentrations that were added, better recoveries of DCL were obtained in the range of 100.28-100.72% w/w with the % RSD ranging from 0.65-0.87 and for RAB it were in range of 99.84-100.05% w/w, with the % RSD ranging from 0.79-1.62.

**Table11: Recovery study data**

| Level of Recovery | Amount present (mg) | | Added concentration | | Amount recovered(mg) | | %Recovery | |
|---|---|---|---|---|---|---|---|---|
| | **DCL** | **RAB** | **DCL** | **RAB** | **DCL** | **RAB** | **DCL** | **RAB** |
| | 10 | 2 | 8 | 1.6 | 18 | 3.56 | 100 | 98.99 |
| **80%** | 10 | 2 | 8 | 1.6 | 18.15 | 3.6 | 100.87 | 100 |
| | 10 | 2 | 8 | 1.6 | 18.23 | 3.62 | 101.3 | 100.55 |
| | 10 | 2 | 10 | 2 | 19.91 | 3.96 | 99.56 | 99.16 |
| **100%** | 10 | 2 | 10 | 2 | 20.26 | 4 | 101.30 | 100 |
| | 10 | 2 | 10 | 2 | 20.04 | 4.02 | 100.2 | 102.34 |
| | 10 | 2 | 12 | 2.4 | 22.09 | 4.40 | 100.43 | 100.16 |
| **120%** | 10 | 2 | 12 | 2.4 | 22.19 | 4.43 | 100.87 | 100.83 |
| | 10 | 2 | 12 | 2.4 | 21.90 | 4.36 | 99.56 | 99.16 |

**Table12: Statistical validation of recovery study data**

| Level of recovery | %Mean recovery | | SD | | %RSD | |
|---|---|---|---|---|---|---|
| | DCL | RAB | DCL | RAB | DCL | RAB |
| **80** | 100.72 | 99.84 | 0.66 | 0.79 | 0.65 | 0.79 |
| **100** | 100.35 | 100.5 | 0.88 | 1.64 | 0.87 | 1.62 |
| **120** | 100.28 | 100.05 | 0.66 | 0.84 | 0.65 | 0.83 |

***Indicates average of three determinations*

Quantification of Diclofenac Sodium and Rabeprazole Sodium in combined tablet dosage form was successfully accomplished by applying the proposed validated method. Figure 4 displays the FTIR spectra obtained from the relevant sample of tablet dosage form which was diluted with potassium bromide. For DCL, the LOD was 2.583% w/w and LOQ was 0.2454% w/w, while for RAB, the the LOD was 7.828% w/w and LOQ was 0.7436% w/w.

**Table13: LOD and LOQ**

| Name of the drug | LOD (%w/w) | LOQ (%w/w) |
|---|---|---|
| **DCL** | 2.583 | 7.828 |
| **RAB** | 0.2454 | 0.7436 |

## Conclusion

The FT-IR spectrophotometric method was developed, validated and found to be suitable for simultaneous estimation of diclofenac sodium and rabeprazole sodium in tablet dosage form. This was accomplished in accordance with the guidelines provided by ICH. Analysis of the combined mixture using the proposed method yielded results that were found to be highly reproducible and reliable. The methods that have been developed are simple while also being sensitive, accurate and precise. Both diclofenac sodium and rabeprazole sodium were subjected to forced degradation studies, and the results of these studies can demonstrate the extent to which degradation occurs under the experimental conditions that were selected. This method is both quick and environmentally friendly, making it suitable for use in the pharmaceutical industry for quality control and routine analysis of finished products. It does not involve the use of hazardous chemicals or solvents and involves relatively simple sample preparation which leads to an economical and environmentally friendly method.

## Acknowledgement

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