

# Development and Validation of UV-Spectrophotometric Methods for Simultaneous Determination of Ibuprofen and Tramadol in its Pure and Pharmaceutical Dosage Forms

PM. Rachmale, SB. Jadhav, PD. Chaudhari, BH. Surwase and AJ. Palande

Department of Pharmaceutical Chemistry, P.E. Society's Modern College of Pharmacy, Sector 21, Nigdi, Pune 44, Maharashtra, India.

## ABSTRACT

Development and validation of four simple, rapid, precise, accurate and sensitive UV spectrophotometric methods for the simultaneous estimation of Ibuprofen and Tramadol in bulk and in tablet dosage form. The methods are based on the measurement of absorbance of Ibuprofen and Tramadol at 264nm and 271nm respectively. The linearity of the calibration curves for Ibuprofen and Tramadol in the desired concentration range is good ( $r^2 = 0.999$ ) by these method. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of proposed methods. These methods were successfully applied to the routine determination of these drugs in bulk and in its pharmaceutical dosage form.

**Keywords:** Ibuprofen; Tramadol; simultaneous determination; area under curve.

## INTRODUCTION

Ibuprofen chemically, (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid Ibuprofen is known to have an antiplatelet effect, though it is relatively mild and somewhat short-lived when compared with aspirin or other better-known antiplatelet drugs. In general, ibuprofen also acts as a vasoconstrictor, having been shown to constrict coronary arteries and some other blood vessels mainly because it inhibits the vasodilating prostacyclin produced by cyclooxygenase 2 enzymes<sup>1</sup>.

Tramadol chemically, (1R,2R)-rel-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol. Tramadol is a very weak  $\mu$ -opioid receptor agonist, induces serotonin release, and inhibits the reuptake of norepinephrine. Tramadol is converted to O-desmethyltramadol, a significantly more potent  $\mu$ -opioid agonist. The opioid agonistic effect of tramadol and its major metabolite(s) is almost exclusively mediated by such  $\mu$ -opioid receptors. This further distinguishes tramadol from opioids in general (including morphine), which do not possess tramadol's degree of receptor subtype selectivity and which are much stronger opiate-receptor agonists<sup>2</sup>.

Literature survey reveals that IBU can be estimated by HPLC<sup>3-9</sup>, spectrophotometry<sup>10-12</sup>, TLC<sup>13</sup> and HPTLC<sup>14-15</sup> methods individually or in combination with other drugs. TRA is reported to be estimated by HPLC<sup>16-17</sup>, spectrophotometry<sup>18</sup> and HPTLC<sup>19</sup> methods

individually or in combination with other drugs. However, there is no analytical method reported for the estimation of IBU and TRA in a combined dosage formulation. Aim of present work was to develop and validate simple, economic, rapid, accurate and precise RP-HPLC method for determination of these drugs in fixed dose combination. The proposed methods was optimized and validated as per the International Conference on Harmonization (ICH) guidelines<sup>20</sup>.

## MATERIALS AND METHODS

### Materials

The bulk drugs of IBU and TRA were obtained as gift samples from Emcure Pharmaceutical Ltd Hinjewadi and Microlab Ltd Bengaluru respectively. All analytical grade chemicals and solvents were purchased from Merck, India.

### Equipment

A UV-1800 Shimadzu spectrophotometer with data processing system was used for all absorbance measurements. UV spectra of reference and sample solutions were recorded in 1 cm quartz cells at a scan speed 100nm per min.

## PROCEDURE

### Preparation of standard stock solutions

Standard stock solutions of IBU and TRA were prepared by dissolving 10mg each accurately

weighed of standard IBU and TRA in methanol and made the volume up to 100ml with same solvent in 100ml volumetric flask. Working standard solutions were prepared by diluting aliquot portion of standard stock solution of each drug to give concentration 400µg/ml and 50µg/ml of IBU and TRA respectively.

#### Calibration curve

Each working standard solution was scanned between the range 200-400 nm. The calibration curves for IBU and TRA were prepared in the concentration range of 5-40µg/ml and 10-40µg/ml respectively.

#### Method I: Simultaneous equation method

In quantitative determination of two drugs by these method two λs that is 264nm as λ<sub>max</sub> of IBU and 271nm as λ<sub>max</sub> of TRA were selected at which both drugs have absorbance. A set of two simultaneous equations were formed using absorptivity coefficient at selected wavelengths.

The concentrations of two drugs in the mixture were calculated using set of two simultaneous equations:

$$C_{IBU} = A_2 \times ay_1 - A_1 \times ay_2 / ax_2 \times ay_1 - ax_1 \times ay_2 \dots\dots (1)$$

$$C_{TRA} = A_1 \times ax_2 - A_2 \times ax_1 / ax_2 \times ay_1 - ax_1 \times ay_2 \dots\dots (2)$$

Where,

$ax_1$  and  $ax_2$  are absorptivities of IBU at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

$ay_1$  and  $ay_2$  are absorptivities of TRA at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

A<sub>1</sub> and A<sub>2</sub> are Absorbances of mixed standard at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

C<sub>IBU</sub> and C<sub>TRA</sub> are concentration of IBU and TRA respectively.

#### Method II: Area under curve method

From spectra, area under the curves was measured in range of 261-267nm and 268-274nm. The absorptivity coefficients were determined for both the drugs at both the wavelength range and following equation were made.

$$A_1 = 0.9571C_{IBU} + 0.3429C_{TRA} \dots\dots (3) (\lambda_{261-267})$$

$$A_2 = 4.041C_{IBU} + 3.7288C_{TRA} \dots\dots (4) (\lambda_{268-274})$$

Where A<sub>1</sub> and A<sub>2</sub> are area under the curve of sample solution at the wavelength range 261-267nm and 268-274nm respectively and C<sub>IBU</sub> and C<sub>TRA</sub> are concentrations of IBU and TRA respectively. The concentrations of both the drugs in the mixture were determined by equation (3) and (4).

#### Method III: First order derivative Method

In quantitative determination of two drugs by these method two λs (first zero cross point) that

is 263nm as λ<sub>max</sub> of IBU and 277nm as λ<sub>max</sub> of TRA were selected at which both drugs have absorbance. A set of two simultaneous equations were formed using absorptivity coefficient at selected wavelengths.

The concentrations of two drugs in the mixture were calculated using set of two equations:

$$C_{IBU} = A_2 \times ay_1 - A_1 \times ay_2 / ax_2 \times ay_1 - ax_1 \times ay_2 \dots\dots (1)$$

$$C_{TRA} = A_1 \times ax_2 - A_2 \times ax_1 / ax_2 \times ay_1 - ax_1 \times ay_2 \dots\dots (2)$$

Where,

$ax_1$  and  $ax_2$  are absorptivities of IBU at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

$ay_1$  and  $ay_2$  are absorptivities of TRA at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

A<sub>1</sub> and A<sub>2</sub> are Absorbances of mixed standard at (λ<sub>1</sub>) and (λ<sub>2</sub>) respectively.

C<sub>IBU</sub> and C<sub>TRA</sub> are concentration of IBU and TRA respectively

#### Method IV: Q-analysis method

In quantitative determination of two drugs by these method two λs that is 264nm as λ<sub>max</sub> of IBU and 271nm as λ<sub>max</sub> of TRA were selected at which both drugs have absorbance. UV spectrum of two drugs intersects with each other at 257 nm (isoblastic point).

The concentrations of two drugs in the mixture were calculated using set of equations.

#### ANALYSIS OF TABLET FORMULATION

Twenty tablet were weighed accurately and content were emptied. A quantity of powder equivalent to 400mg IBU and 50mg TRA was weighed, transferred to 100ml volumetric flask, dissolved in 100ml methanol. The mixture was ultrasonicated for 20 min. The solution was filtered through whatmann filter paper no. 41 and suitably diluted with methanol to have 400 µg/ml and 50µg/ml of IBU and TRA respectively. Samples were analysed by the proposed methods.

#### RECOVERY STUDIES

The accuracy of proposed methods was checked by recovery study by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 400µg/ml of IBU and 50µg/ml of TRA for all the methods.

#### RESULT AND DISCUSSION

Literature survey reveals not a single method has been reported for analysis of the IBU and TRA by UV spectrophotometric method. So,

the proposed methods for estimation of IBU and TRA in combined dosage form were found to be new, simple, rapid, accurate and economic.

For all the methods, linearity was observed in the concentration range of 5-40 $\mu$ g/ml and 10-40 $\mu$ g/ml for IBU and TRA respectively. Marketed brand of tablet was analysed and amount of drug determined by proposed method ranges from 99 to 101% as shown in table no 1. The proposed method was validated as per ICH guidelines. The accuracy of method was determined at 80, 100 and 120% level. The percentage recovery ranges

from 98 to 101% for all methods. Precision was calculated as interday and intraday variations (% RSD is minimum) for both drugs. These four methods can be successfully used for simultaneous estimation of IBU and TRA in combined dosage form.

### CONCLUSION

The proposed methods have proved to be simple, rapid, precise, accurate sensitive and economical and are suitable for simultaneous quantification of IBU and TRA in bulk and in pharmaceutical dosage forms.

**Table 1: Statistical parameters**

Parameters		IBU	TRA
Linearity range		5-40 $\mu$ g/ml	10-40 $\mu$ g/ml
Slope		0.0196	0.0153
Intercept		0.004	0.0072
Correlation Co-efficient		0.9996	0.9977
Ruggedness	LOD	0.75	1.19
	LOQ	2.27	3.678
Intraday Precision (% R.S.D.)		0.26	0.0815
Interday Precision (% R.S.D.)		0.76	0.501
Robustness (% R.S.D.)		0.501	0.26

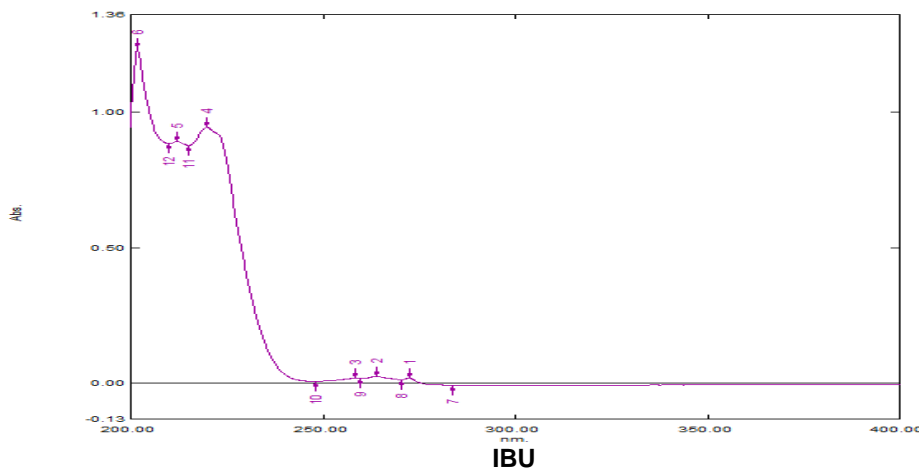
IBU – Ibuprofen, TRA-Tramadol

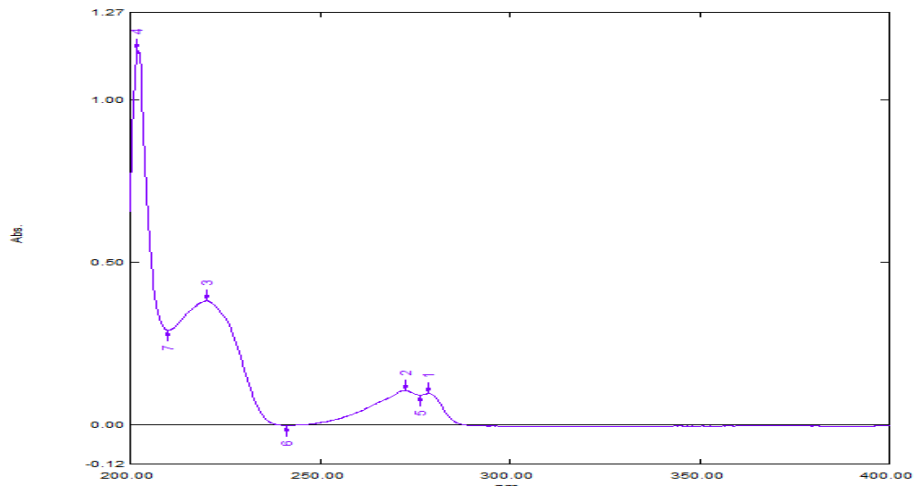
**Table 2: Analysis of tablet formulations**

Method	Formulation	Label claim mg/dose		Amount found mg/dose		%Recovery $\pm$ SD*
Simultaneous Equation Method	Tablet	400	50	399.1	50.05	99.87% $\pm$ 0.2533
Area Under Curve Method	Tablet	400	50	398.08	50.02	99.50% $\pm$ 0.445
First order derivative method	Tablet	400	50	400.119	49.718	99.70% $\pm$ 0.483
Q-analysis method	Tablet	400	50	399.12	50.227	100.1% $\pm$ 0.415

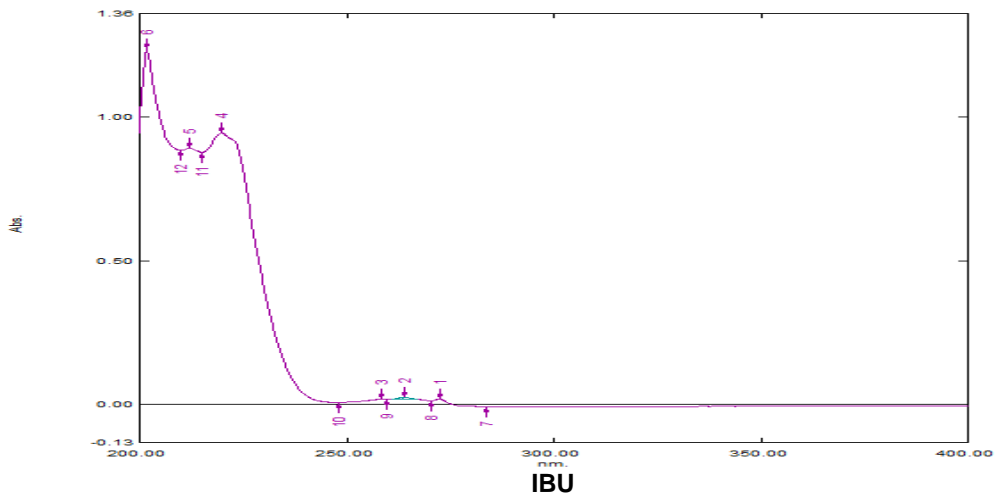
Tablet formulation containing IBU 400 mg and TRA 50 mg per dosage.

\*= Average of 6 determinations

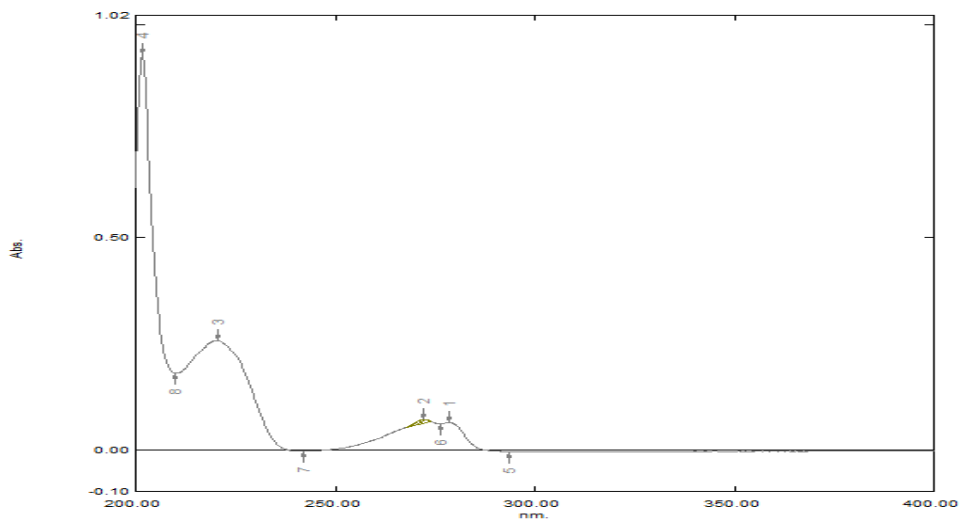




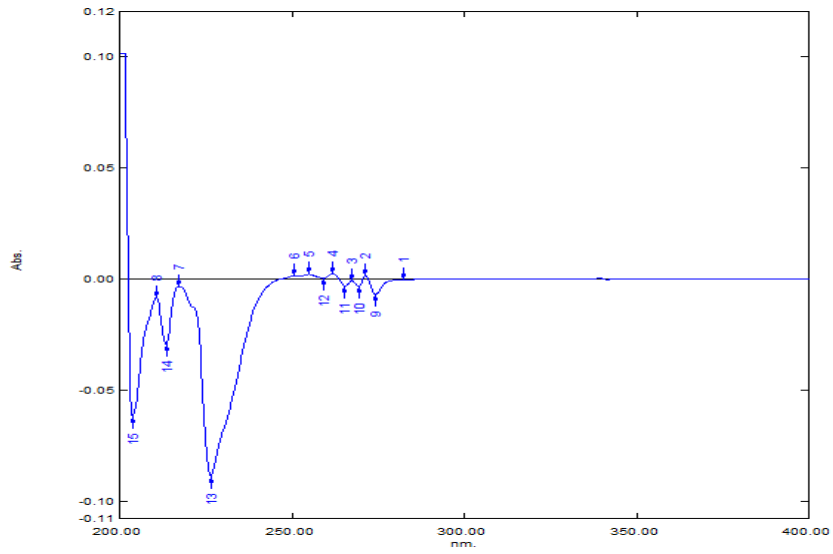
TRA  
Fig. 1: UV Spectrum



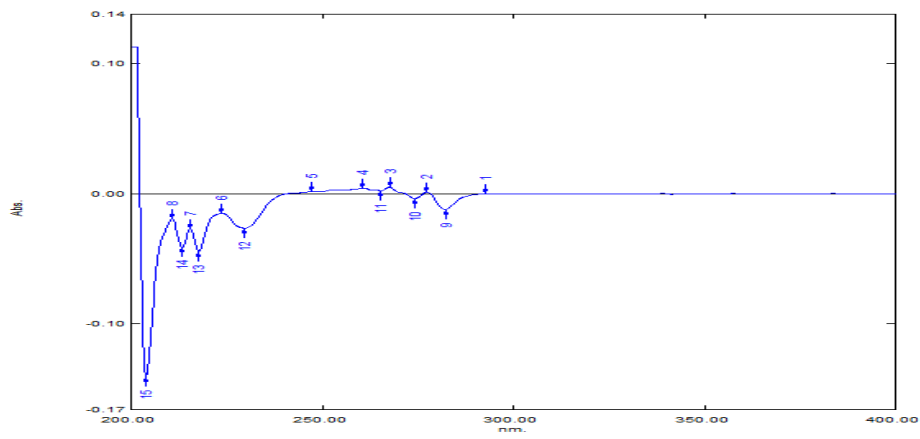
IBU



TRA  
Fig. 2: AUC spectra



IBU



TRA

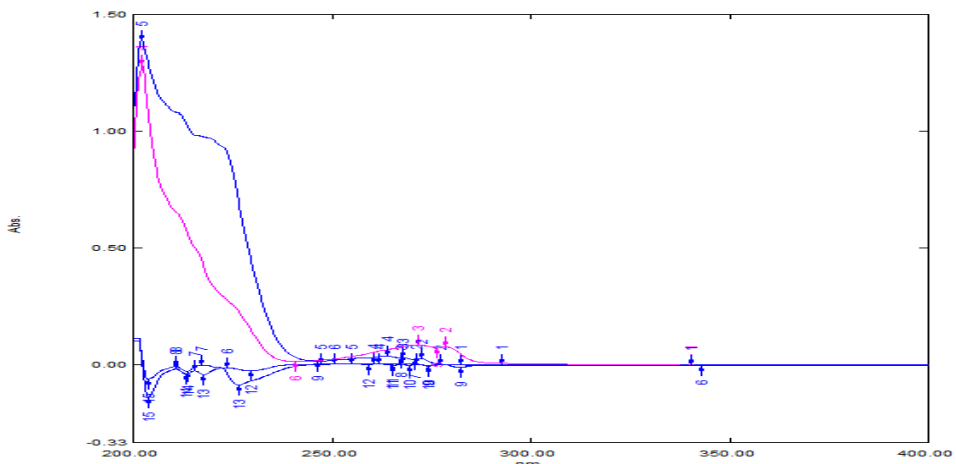


Fig. 3: First order derivative spectra of Mixture

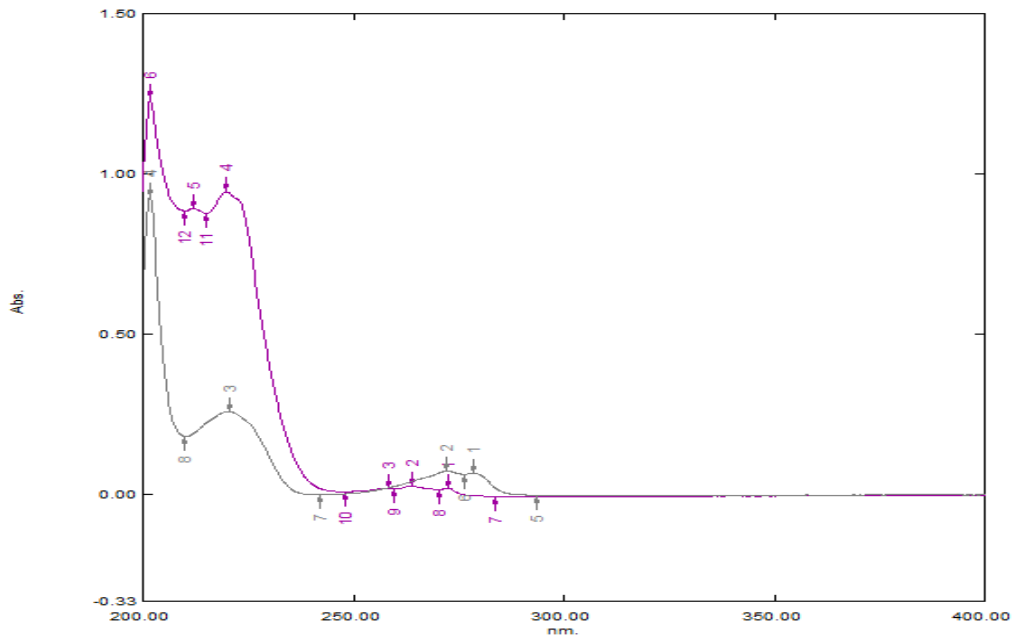
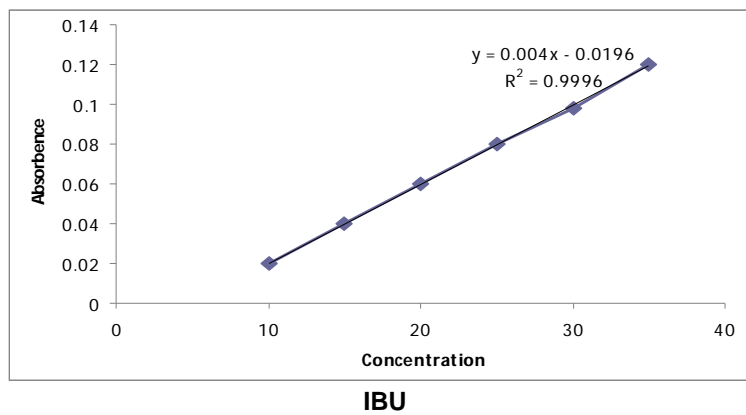


Fig. 4: Q-analysis spectra



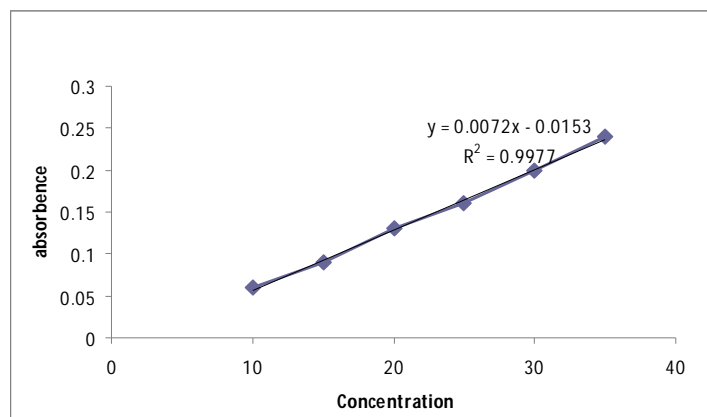


Fig 5: Calibration curve of TRA

## REFERENCES

1. Maryadele JO. The Merck Index, 14th ed., White house Station, NJ, USA: Merck and Co. Inc. 2006;1603.
2. Maryadele JO. The Merck Index, 14th ed., White house Station, NJ, USA: Merck and Co. Inc. 2006;918.
3. Del GK, Aprile S and Grosa G. Forced degradation study of ibuprofen: Characterization of its degradation products. *J Pharm Biomed Anal.* 2012;61:215-223.
4. Umarkar AR, Rewatkar NS, Charde MS, Charde RM and Kasture AV. RP-HPLC method development and validation for estimation of ibuprofen and paracetamol in bulk and capsule dosage forms. *J Pharm Res.* 2011;4(5):1307-1308.
5. Walash M, Belal F, Eid M and Abass S. Simultaneous HPLC determination of tramadol and diclofenac sodium in their combined dosage forms. *J Chromatogr Sci.* 2011;49:159-164.
6. Sahoo M, Syal P, Ingale S, Ingale K, Sindhe S, Sali M, Choudhari VP and Kuchekar BS. Development and validation of a RP-HPLC-PDA method for simultaneous determination of ibuprofen and paracetamol in pharmaceutical dosage form and its application for dissolution study. *Int J Res Pharm Sci.* 2011;2(1):1-7.
7. Modi MV, Patel MM and Patel CN. Development and validation of analytical method for the determination of ibuprofen and paracetamol in pharmaceutical dosage form by RP-HPLC. *Int J ChemTech Res.* 2011;3(3):1259-1264.
8. Kumar S, Joshi A, Thakur R, Pathak AK and Shah K. Simultaneous estimation of Dexibuprofen and paracetamol by RP-HPLC method in combined dosage forms. *Acta Pol Pharm.* 2011;68(6):839-845.
9. Bhavsar SM, Patel DM, Khandhar AP and Patel CN. Validated RP-HPLC method for simultaneous estimation of Diphenhydramine hydrochloride and ibuprofen in solid dosage form. *J Chem Pharm Res.* 2010;2(2):563-572.
10. Nikhade RV, Thakur AD, Choudhari SB and Chandewar AV. Simultaneous estimation of famotidine and ibuprofen by UV spectrophotometer using multicomponent mode method. *J Pharm Res.* 2011;4(7):2297-2299.
11. Gupta KR and Joshi RR. Simultaneous UV-Spectrophotometric determination of ibuprofen and paracetamol in pharmaceutical formulation. *Pharm Sin.* 2010;1(2):44-51.
12. Choudhari VP, Chabukswar AR, Tryambake MU and Savakhande SN. Spectrophotometric simultaneous determination of pyrimethamine and ibuprofen in combined tablet dosage form by absorption corrected method and area under curve method. *Int J Pharm Sci Rev Res.* 2011;7(2):182-185.
13. Ragehy NA, Ellaithy MM and El-Ghobashy MA. Determination of ibuprofen in its binary mixtures by tlc/densitometry. *IL Farmaco.* 2003;58:463-468.
14. Dhaneshwar SR, Bhusari VK and Patil ST. Validated HPTLC method for simultaneous estimation of ibuprofen

- and famotidine in bulk drug and formulation, *Int J Pharma Bio Sci.* 2011;2(2):482-490.
15. Kuchekar BS, Sahoo M, Syal P, Hable AA, Raut RP and Chaudhari VP. Development and validation of HPTLC method for simultaneous estimation of ibuprofen and paracetamol in combined dosage form, *Pharmaceutical methods.* 2011;2(3):178-183.
  16. Rao JR, Mulla TS, Yadav SS, Bharekar VV and Rajput MP. Development and validation of HPLC method for tramadol in bulk drug and formulation, *Pharm. Globale.* 2011;2(7):1-4.
  17. Pokharkar DV, Korhale R, Jadhav S, Birdar N, Puri DC and Wani NP. Stability indicating RP-HPLC-PDA method for stability indicating assay method for tramadol and paracetamol from tablet dosage form, *Pharmacia Lettre.* 2011;3(6):49-57.
  18. Kothapalli LP, Karape AK, Thomas AB, Nanda RK, Gaidhani P and Choudhari ME Simultaneous spectrophotometric estimation of tramadol and paracetamol trometamol in pharmaceutical dosage form. *Pharma Chem.* 2011;3(1):365-371.
  19. Rao JR, Mulla TS, Yadav SS, Bharekar VV and Rajput MP. Simultaneous HPTLC determination of tramadol and paracetamol in pharmaceutical dosage form. *Pharma Chem.* 2011;3(3):32-38.
  20. ICH, Q2(R1), validation of analytical procedures: Text and Methodology International Conference on Harmonization, Geneva, Switzerland, 2005.