

**APPLICATION OF HPTLC FOR SIMULTANEOUS ESTIMATION OF ZALTOPROFEN
AND PARACTAMOL IN COMBINED DOSAGE FORM.**

N-K_Zahira*¹ and P-N_Presannakumaran¹

¹College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala 695011.

*Corresponding Author: N-K_Zahira

College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala 695011.

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ABSTRACT

A simple, sensitive HPTLC method has been developed for the estimation of Zaltoprofen and Paracetamol in combined dosage form. The stationary phase was pre-coated plates of silica gel G 60 F254 and the mobile phase used was Toluene: Isopropyl alcohol in the ratio 5:2. The R_f values were found to be 0.59 for Paracetamol and 0.78 for Zaltoprofen respectively. The plate was scanned and quantified at 254 nm. The calibration curve of Paracetamol and Zaltoprofen were prepared and it was found to be linear in the range of 200- 500 ng/2 μ l and 100-400 ng/2 μ l respectively. The correlation coefficient was found to be 0.9913 (Area wise) and 0.9932 (Height wise) for Paracetamol and 0.9907 (Height wise) and 0.9902 (Area wise) for Zaltoprofen. The percentages of label claim for Paracetamol were found to be 99.9654% (Height wise) and 100.066% (Area wise). Percentages of label claim for Zaltoprofen were found to be 100.0145% (Area wise) and 100.0644% (Height wise). The suggested methods were validated in compliance with the ICH guidelines and were successfully applied for determination of ZLT and PAR in their laboratory prepared mixture and commercial Tablet. This is a simple manipulation with maximum reproducibility and robustness, which can be used for the analysis of binary mixture with overlapped spectra for routine QC testing.

KEYWORDS: Chromatography, Binary mixture, Paracetamol, Zaltoprofen.

INTRODUCTION

Zaltoprofen (ZLT) is a non-steroidal anti-inflammatory drug¹. The chemical name of ZLT is (\pm)-2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2-yl) propionic acid. CAS No is 747411-43-6. Zaltoprofen selectively inhibits cyclooxygenase-2 activity and prostaglandin E2 production. It is used in the treatment of Rheumatoid arthritis, Osteoarthritis, and other chronic inflammatory Pain conditions ZLT is a unique compound that also has anti-bradykinin activity.^[1]

Paracetamol (PAR) Paracetamol or acetaminophen, chemically named as N-acetyl-p aminophenol, is a widely used analgesic and antipyretic (fever reducer).

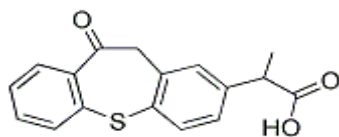


Fig. 1: Structural formula of Zaltoprofen.

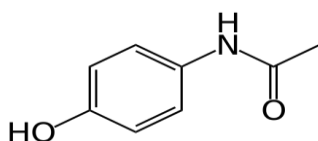


Fig. 2: Structural formula of paracetamol.

Zaltoprofen is marketed in combination with Paracetamol under the Trade Name REDUCIN A by JB Chemicals & Pharmaceuticals Ltd, Mumbai. Literature reveals that there are many methods for the individual determination of Zaltoprofen and Paracetamol; but to the best of my knowledge no methods are cited for the determination of Zaltoprofen and Paracetamol in combined dosage. So, it was proposed to develop an economical, rapid and simple HPTLC method for the simultaneous estimation of these drugs in combined dosage forms.^[2,3,4,5]

Theory of the Proposed Methods

Thin Layer Chromatography (TLC) is a form of adsorption chromatography in which the adsorbent is spread in a thin layer on a glass plate or plastic sheet, held in place by a chemical binder. The different components of the sample are separated by their interaction with the stationary phase and the liquid mobile phase that moves along the stationary phase. High-Performance Thin Layer Chromatography (HPTLC) is a form of Thin Layer Chromatography (TLC) that provides superior separation power using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning and improved sample application. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of

mobile phase, and efficient data acquisition and processing. The term HPTLC is used for the technique in which substances are accurately and precisely assayed using high performance grades of silica gel. In HPTLC, the sorbent material like silica gel G60 has finer particle size distribution than conventional TLC material. It is a powerful, reliable and cost effective method for qualitative and quantitative analysis.^[6,7,8,9]

In HPTLC, the mobile phase moves through the pre-coated stationary phase by capillary action or by gravity. The basic chromatographic measurement of a substance in HPTLC is the R_f value.^[10,11,12]

$$R_f = \frac{\text{Distance travelled by the solute from the line of application of the solute}}{\text{Distance travelled by the solvent from the line of application of the solute}}$$

MATERIALS AND METHODS

Reagents and chemicals

1. Methanol HPLC grade obtained from S D Fine - Chemicals Limited, Mumbai
2. Paracetamol RS
3. Zaltoprofen RS
4. Marketed combination product of Paracetamol and Zaltoprofen (REDUCIN A by JB Chemicals and Pharmaceuticals Ltd
5. Toluene HPLC grade obtained from Ranbaxy Fine-Chemicals Limited, New Delhi
6. Isopropyl Alcohol

EQUIPMENT USED.

Application Mode: CAMAG Linomat IV
 Development Mode: CAMAG Twin Through chamber
 Scanner: TLC scanner with WINCATS software
 Visualization: CAMAG UV Cabinet
 Quantification: CAMAG Video Densitometer
 Stationary phase: HPTLC pre coated plates, silica gel G60 F254 (20x20).

Pure sample

Zaltoprofen RS was kind gift from Intas Pharmaceuticals, Assam. Paracetamol RS was purchased from Indian Pharmacopoeia Commission, Ghaziabad. Purity of the compounds was checked according to the pharmacopoeia specification.

Pharmaceutical dosage form

REDUCIN A (Paracetamol 325mg and Zaltoprofen 80 mg) Manufactured by JB Chemicals and Pharmaceuticals (P) Ltd, Mumbai was obtained from the local market.

Methodology Adopted

1. Preparation of standard solutions
2. Development of solvent system
3. Development of chromatogram
4. Determination of R_f values of Paracetamol and Zaltoprofen
5. Preparation of calibration curves of Paracetamol and Zaltoprofen and analysis of combined tablet dosage form

6. Validation of the proposed method.

Preparation of standard solutions

• Stock solution of Paracetamol

Weighed accurately 50 mg of Paracetamol RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made up to the volume. This solution had a concentration of 1mg/ml.

• Stock solution of Zaltoprofen

Weighed accurately 50 mg of Zaltoprofen RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made up to the volume. This solution had a concentration of 1mg/ml.

• Stock solution of standard drug mixture

50 mg of Paracetamol RS, 50 mg of Zaltoprofen RS were weighed separately and transferred into a 50ml standard flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made up to the mark with methanol to obtain a mixture with concentration of 1000µg/ml of Paracetamol and 1000 µg/ml of Zaltoprofen (Solution A). 4 ml of solution 'A' was accurately pipetted out in to a 10 ml standard flask and made up to the volume with methanol to get a concentration of 400µg/ml Paracetamol and 400µg/ml Zaltoprofen respectively (Solution B).

Development of solvent system

The mobile phase was selected based on the polarity of analytes (Paracetamol and Zaltoprofen) and absorption property of silica gel plates. The solubility of drugs also played a significant role in the selection of suitable solvent system. The suitable solvent system was selected by a series of trial and error process. Different solvent systems were used in different proportions and the summary is listed in Table (6.1).

By the trial and error process, a solvent system of Toluene: IPA in the ratio 5:2 was selected for the HPTLC analysis of Paracetamol and Zaltoprofen in the combined form.

Development of chromatogram

• Selection of chromatographic layer

HPTLC pre-coated plate of silica gel G 60 F254 was employed for the spotting of standard solutions.

• Preparation of mobile phase and saturation of Twin trough chamber

A 10 ml mobile phase (Toluene: IPA in the ratio 5:2) was freshly prepared and transferred in to a clean and dried twin trough chamber. The chamber was then allowed to saturate for 30 minutes.

• Activation of plate and sample application

Two tracks were selected on the activated pre coated HPTLC plate and spotting was done by using CAMAG Linomat IV automatic sample applicator in the form of bands. Paracetamol standard was applied on the first

track, Zaltoprofen on the second track. Volume of sample application was selected according to the volatility of solvent used for preparing the sample solution.

The applied band was sharp when the volume was 2 μ l. A band width of 4mm was selected for the entire experiment.

The following manual adjustments were done in the Linomat applicator

| | |
|--------------------|------------------|
| Plate size | : 10 x 5cm |
| Start Position | : 12mm |
| Band width | : 4mm |
| Application volume | : 2 μ l |
| Flow rate | : 2 μ l/sec. |
| Space | : 12mm |

After application the plate was taken out dried using a hair drier and the position of spots was visualized and confirmed under UV cabinet at 254nm.

• Development of spot

The plate was developed in the saturated twin trough chamber containing the mobile phase. The plates were dried after development and viewed under UV lamp to evaluate the spots obtained. The spots were uniform and there was no tailing.

Determination of R_f values of Paracetamol and Zaltoprofen

• Detection and visualization

The developed plate was mounted on the CAMAG HPTLC scanner IV and scanned from 200 – 400 nm. The spots showed good response at 254 nm. The R_f values are furnished in Table (6.2) and the chromatograms were displayed in Figures (6.1, and 6.2).

Preparation of calibration curves of Paracetamol and Zaltoprofen and analysis of combined tablet dosage form.

A. Preparation of standard solutions

• Standard solution of Paracetamol RS in methanol

Weighed accurately 50 mg of Paracetamol RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made up to the volume. This solution had a concentration of 1000 μ g/ml. From the above solution 4ml, 6ml and 8ml were pipetted out in to three numbered 10ml standard flask and volume was made up to the mark with methanol to get a concentration of 400 μ g/ml, 600 μ g/ml and 800 μ g/ml.

• Standard solution of Zaltoprofen RS in methanol

Weighed accurately 50 mg of Zaltoprofen RS and transferred to a 50ml standard flask. It was dissolved in HPLC grade methanol and made up to the volume. This solution had a concentration of 1mg/ml.

• Stock solution of standard drug mixture

50 mg of Paracetamol RS, 50 mg of Zaltoprofen RS were

weighed separately and transferred into a 50ml standard flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made up to the mark with methanol to obtain a mixture with concentration of 1000 μ g/ml of Paracetamol and 1000 μ g/ml of Zaltoprofen (Solution A). 4 ml of solution 'A' was accurately pipetted out in to a 10 ml standard flask and made up to the volume with methanol to get a concentration of 400 μ g/ml Paracetamol and 400 μ g/ml Zaltoprofen respectively (Solution B).

B. Preparation of sample solution

Details of Analyzed Dosage form

| | |
|---------------------|---|
| Trade name | : REDUCIN A |
| Each tablet contain | : Paracetamol 325mg Zaltoprofen 80 mg |
| Batch No. | : XRX 2001 |
| Mfg. Date | : JUL. 2012 |
| Exp. Date | : AUG. 2014 |
| Mfd. By | : JB Chemicals and Pharmaceuticals (P) LTD |

Twenty tablets of REDUCIN A were weighed; average weight of one tablet was determined and finely powdered with the help of mortar and pestle. A quantity of powder equivalent to 50 mg of Paracetamol and 12.08 mg of Zaltoprofen was accurately weighed, transferred to a stoppered flask and extracted with 20ml of methanol initially by shaken vigorously for 15 minutes. The solution was transferred to a 50 ml standard flask through a Whatman No. 1 filter paper. The residue was then further extracted twice with 10 ml methanol and transferred to the same standard flask through the same filter paper. The volume was finally made up to 50 ml with methanol. The resulting solution had a concentration of 1000 μ g/ml of Paracetamol and 241.6 μ g/ml of Zaltoprofen as per label claim. (Solution A). 4 ml of solution 'A' was accurately pipetted out in to a 10 ml standard flask and made up to the volume with methanol to get a concentration of 400 μ g/ml Paracetamol and 350 μ g/ml of Zaltoprofen respectively (Solution B).

C. Development of chromatogram

• Selection of chromatographic layer

HPTLC pre-coated plate of silica gel G 60 F254 was employed for the spotting of standard solutions.

• Preparation of mobile phase and saturation of Twin trough chamber

A 10 ml mobile phase (Toluene: IPA in the ratio 5:2) was freshly prepared and transferred in to a clean and dried twin trough chamber. The chamber was then allowed to saturate for 30 minute.

• Activation of plate and sample application

Fourteen tracks were selected on the activated pre coated HPTLC plate and spotting was done by using CAMAG Linomat IV automatic sample applicator in the form of bands. Paracetamol standard was applied on the first four

tracks, Zaltoprofen on the second four tracks. Standard drug mixture on next track and sample on next track.

The following manual adjustments were done in the Linomat applicator

| | |
|--------------------|------------------|
| Plate size | : 20 x 10cm |
| Start Position | : 10mm |
| Band width | : 4mm |
| Application volume | : 2 μ l |
| Flow rate | : 2 μ l/sec. |
| Space | : 9 mm |

After application the plate was taken out, dried using a hair drier and the position of spots were visualized and confirmed under UV cabinet at 254nm.

• Development of spot

The plate was developed in the saturated twin trough chamber containing the mobile phase. The plate was

dried after development and viewed under UV lamp to evaluate the spots obtained. The spots were uniform without tailing.

• Scanning and integration of chromatogram

The developed plate was mounted on the CAMAG HPTLC scanner IV and scanned at 254 nm. The results are furnished in Table (6.3). The calibration graphs of peak area v/s concentration and peak height v/s concentration were plotted and shows in Figures (6.3) to (6.6). The overlay spectrum is shown in Figures (6.7) and (6.8). Chromatograms of standards and sample are displayed in Figures (6.9) to (6.18). The developed plate is shown in Figure (6.19).

RESULTS AND DISCUSSION

Development of solvent system

Table 6.1 Solvent system selection – trial and error – data

| Sl. No | Solvent system | Ratio | Inference |
|--------|---------------------|------------|---------------------------------------|
| 1 | Toluene | 10:0 | No separation o |
| 2 | Methanol | 10:0 | High R_f value |
| 3 | Isopropyl alcohol | 10:0 | High R_f value |
| 4 | Toluene :IPA | 10:1 | High R_f value |
| 5 | Toluene :IPA | 5:5 | High R_f value |
| 6 | Toluene :IPA | 5:2 | R_f:0.59 and 0.28 |

Determination of R_f values of Paracetamol and Zaltoprofen

Table 6.2: R_f values of drugs under study.

| DRUGS | R_f |
|-------------|-------|
| Paracetamol | 0.59 |
| Zaltoprofen | 0.78 |

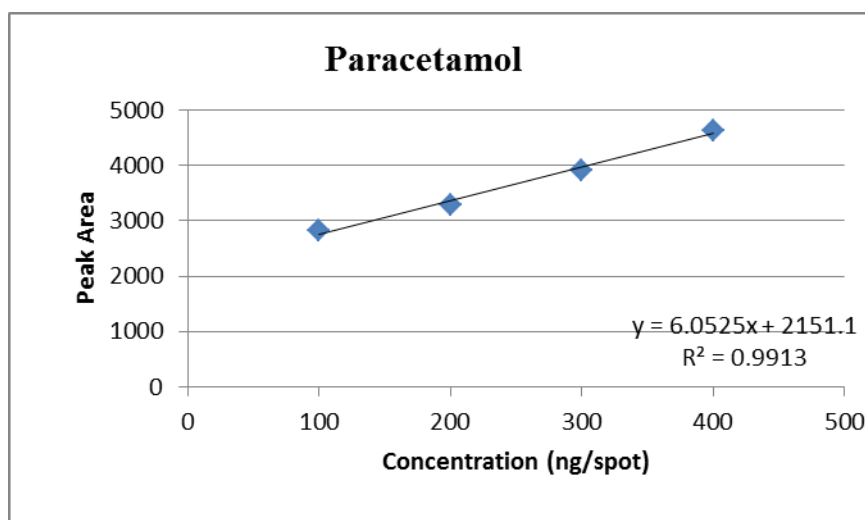


Fig. 6.1: Linearity of Paracetamol.

Table 6.3: Chromatogram Analysis Data.

| Track | Drug | Amount Fraction (ng) | R _f | Peak Height | Peak Area (AU) |
|-------|-------------|----------------------|----------------|-------------|----------------|
| 1 | P | 200 | 0.59 | 151.2 | 2821.9 |
| 2 | P | 300 | 0.59 | 172.2 | 3291 |
| 3 | P | 400 | 0.59 | 199.8 | 3911.4 |
| 4 | P | 500 | 0.59 | 230.5 | 4632.6 |
| 5 | Z | 100 | 0.78 | 71.8 | 1727.7 |
| 6 | Z | 200 | 0.78 | 129.8 | 3337.2 |
| 7 | Z | 300 | 0.78 | 210.3 | 5697.6 |
| 8 | Z | 400 | 0.78 | 257.1 | 7066.1 |
| 9 | Std Mixture | P-400 | 0.59 | 229.1 | 4579.6 |
| 10 | | Z-200 | 0.78 | 135.7 | 3539.9 |
| 12 | Sample | P | 0.59 | 230.4 | 4567.9 |
| 14 | | Z | 0.78 | 136.1 | 3542.5 |

RESULTS

Each tablet contains (label claim),

Paracetamol: 325 mg

Zaltoprofen: 80 mg

Average weight of one tablet: 0.58915g

Weight equivalent to 50 mg of PAR

and 12.30 mg of ZLT: 0.0906384g

Weight taken: 0.090g

Average content of Paracetamol per tablet by proposed method,

Height wise: 99.9654%

Area wise: 100.066%

Average content of Zaltoprofen per tablet by proposed method,

Height wise: 100.0644%

Area wise: 100.0145%

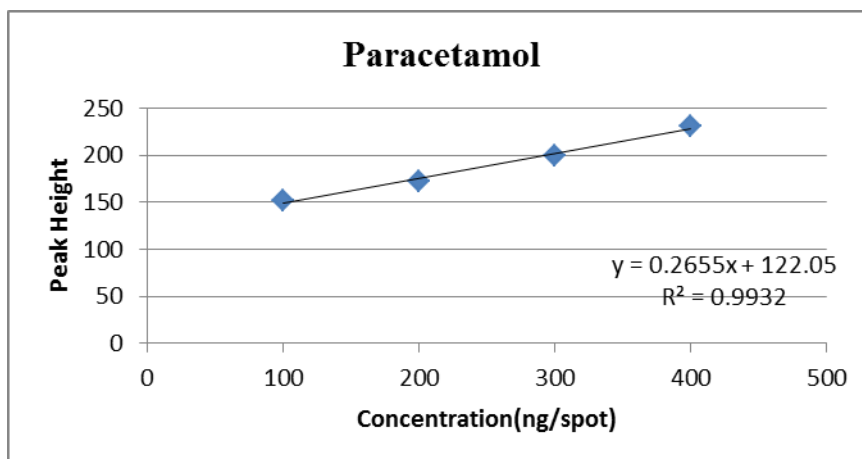


Fig. 6.2: Linearity of Paracetamol.

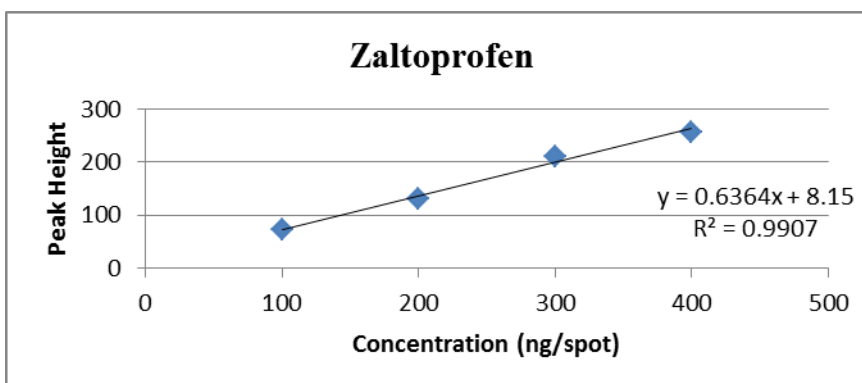


Fig. 6.3: Linearity of Zaltoprofen.

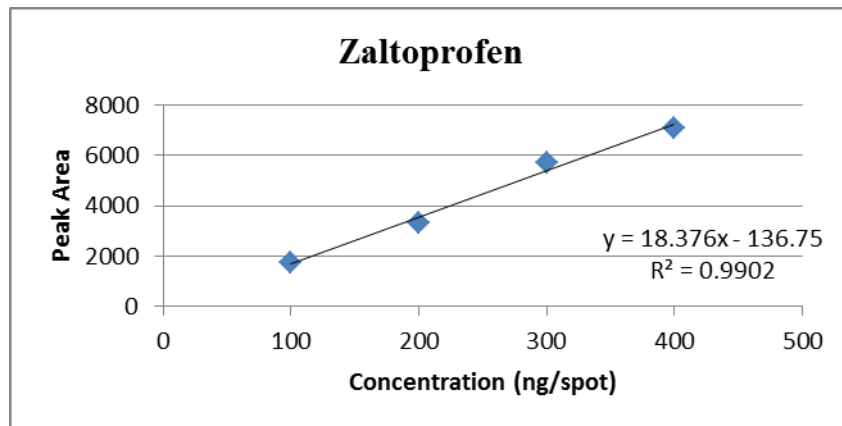


Fig. 6.4: Linearity of Zaltoprofen.

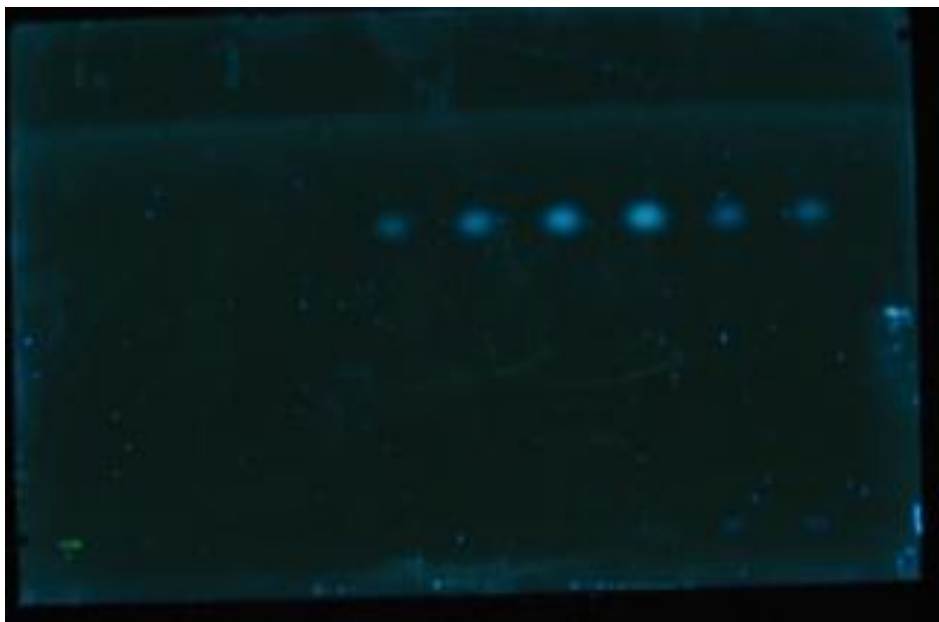


Fig. 6.5: Photograph of HPTLC plate.

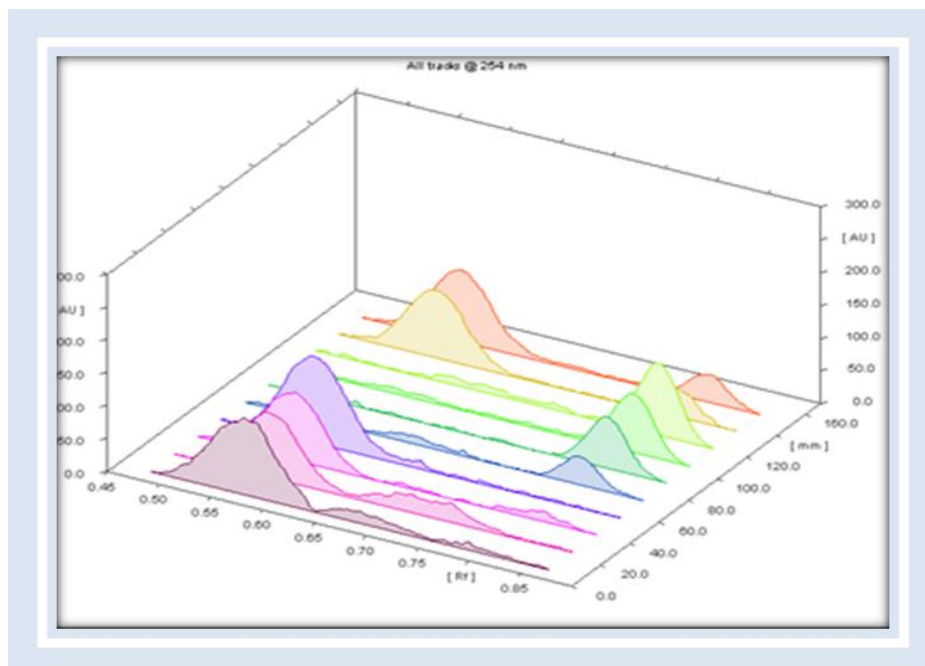


Fig. 6.6(a): 3D Spectrum of Chromatographic Plate.

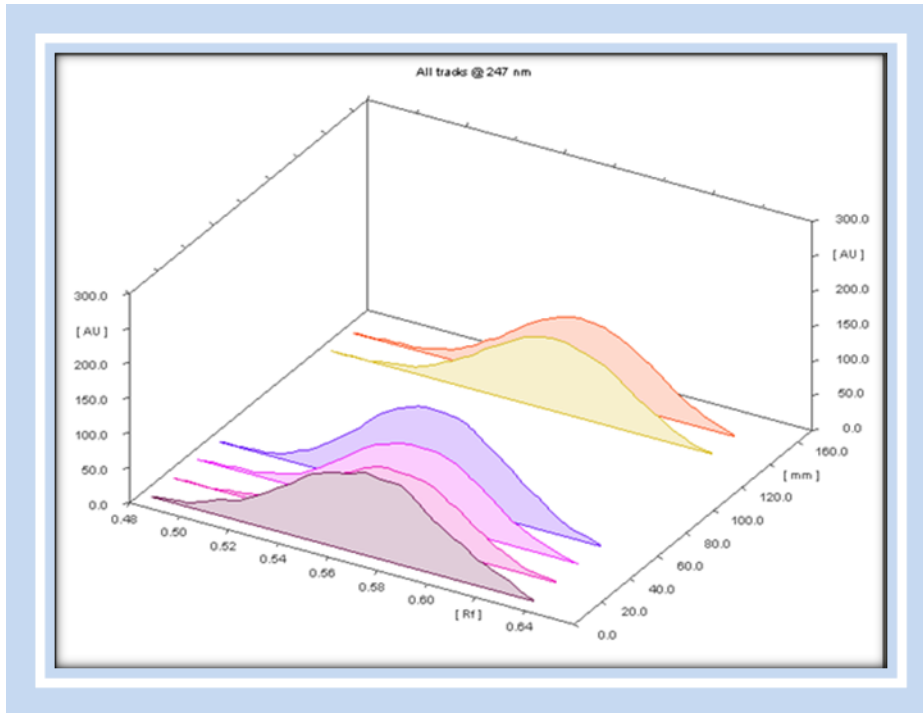


Fig. 6.6(b): 3D Spectrum of Chromatographic Plate.

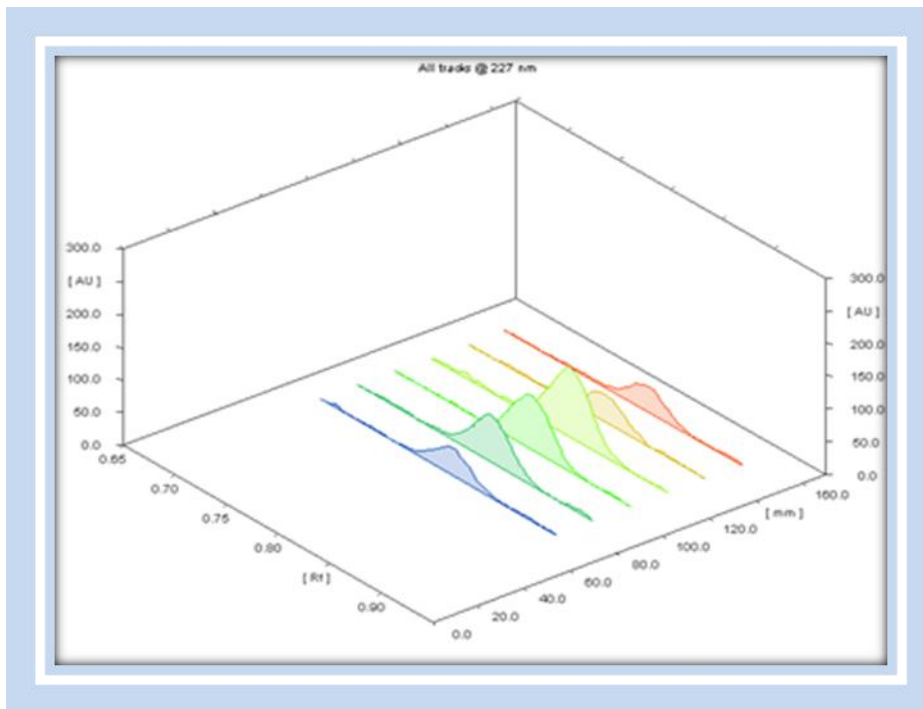


Fig. 6.6(c): 3D Spectrum of Chromatographic Plate.

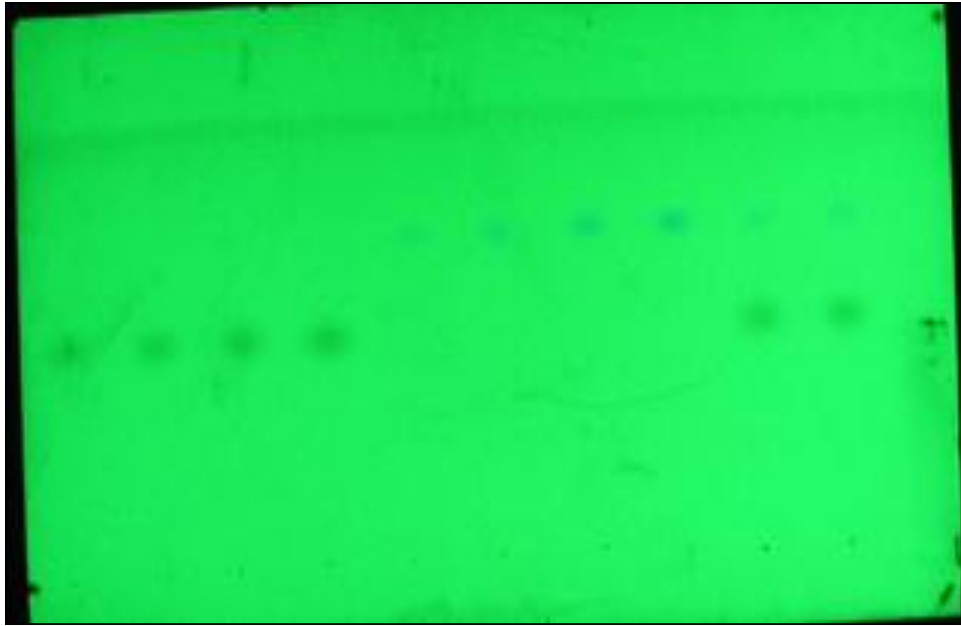
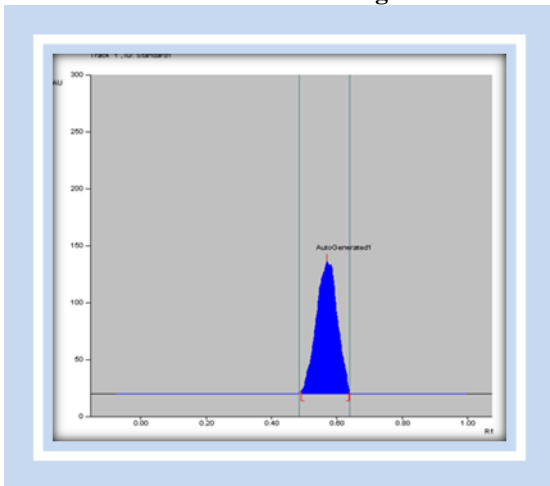
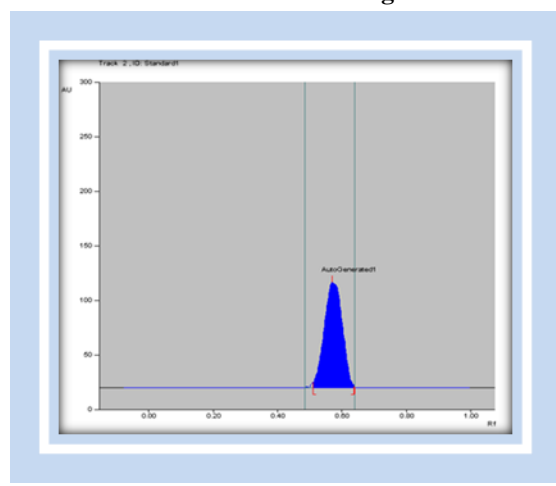


Fig. 6.7: HPTLC chromatogram of Paracetamol.

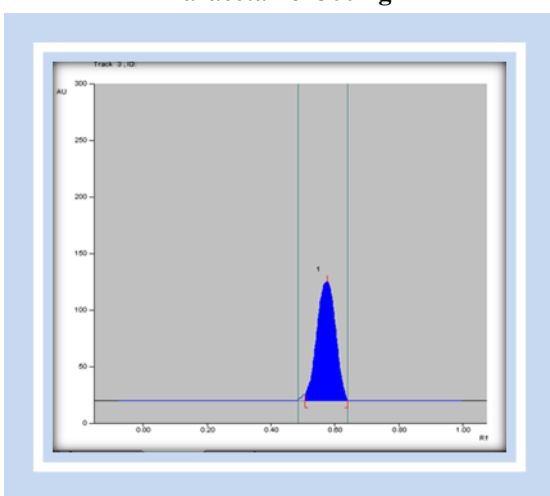
Paracetamol 100ng



Paracetamol 200ng



Paracetamol 300 ng



Paracetamol 400ng

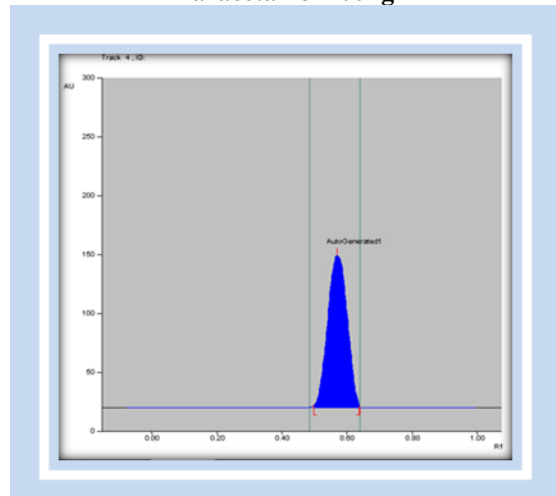


Fig. 6.8: HPTLC Chromatogram of Zaltoprofen.

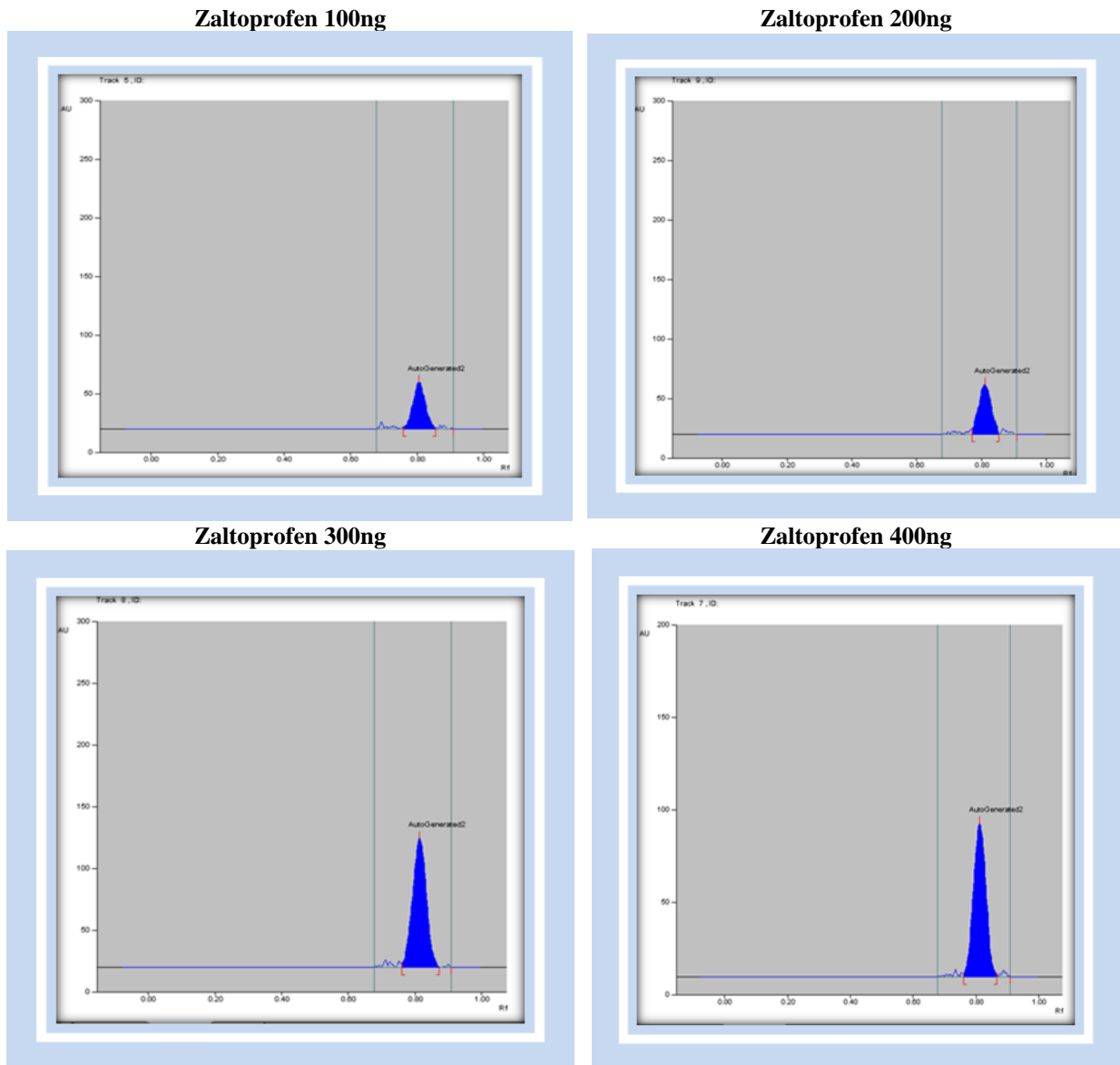


Fig. 6.9: HPTLC Chromatogram of standard mixture.

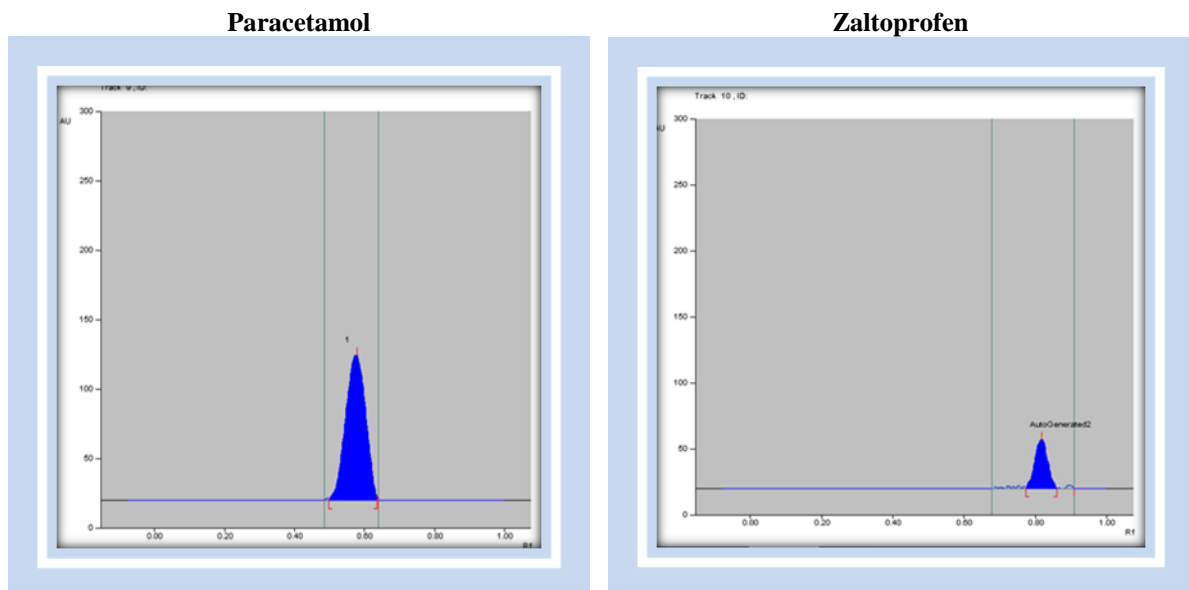


Fig. 6.10: HPTLC Chromatogram of standard

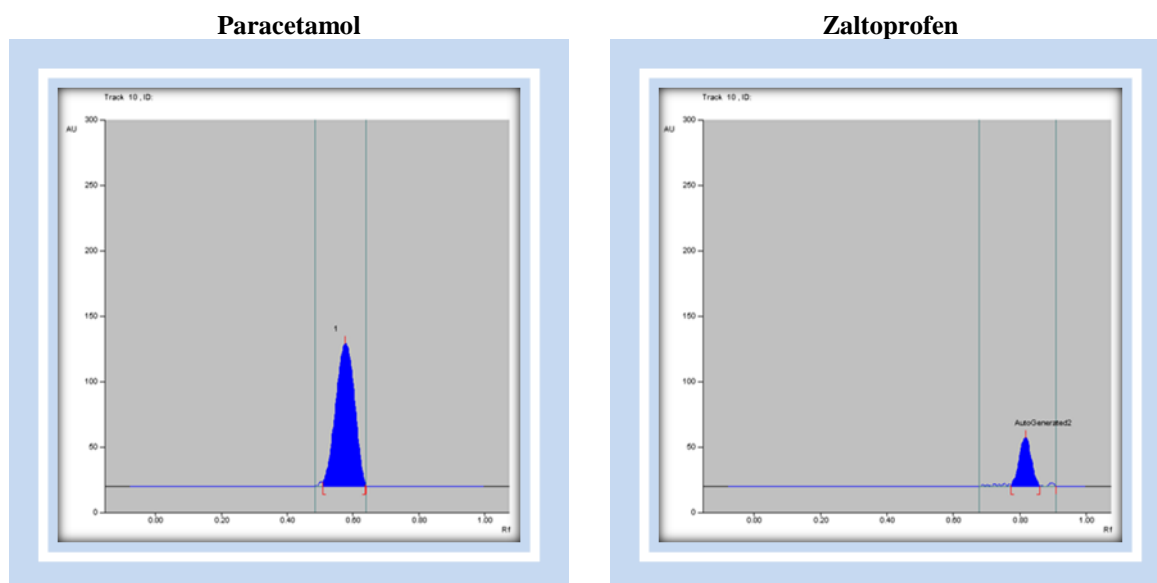


Fig. 6.11: HPTLC Chromatogram of sample.

A simple, sensitive HPTLC method has been developed for the estimation of Zaltoprofen and Paracetamol in combined dosage form. The stationary phase was pre-coated plates of silica gel G 60 F254 and the mobile phase used was Toluene: Isopropyl alcohol in the ratio 5:2.

The R_f values were found to be 0.59 for Paracetamol and 0.78 for Zaltoprofen respectively. The plate was scanned and quantified at 254 nm. The calibration curve of Paracetamol and Zaltoprofen were prepared and it was found to be linear in the range of 200- 500 ng/2 μ l and 100-400 ng/2 μ l respectively. The correlation coefficient was found to be 0.9913 (Area wise) and 0.9932 (Height wise) for Paracetamol and 0.9907 (Height wise) and 0.9902 (Area wise) for Zaltoprofen.

- The Paracetamol content in dosage forms was estimated by the proposed method and were found to be 325.2145 mg (Area wise) and 324.8876mg (Height wise)
- The Zaltoprofen content in dosage forms was estimated by the proposed method and were found to be 80.0116 mg (Area wise) and 80.05154 mg (Height wise) and the result was found to be in agreement with the label claim.
- The percentages of label claim for Paracetamol were found to be 99.9654% (Height wise) and 100.066% (Area wise).
- Percentages of label claim for Zaltoprofen were found to be 100.0145% (Area wise) and 100.0644% (Height wise)

The HPTLC method demonstrated in this work are applicable to the estimation of Zaltoprofen and Paracetamol in the combination tablet dosage form. In order to ensure that the data generated with the above methods are accurate and precise, the experiments have been performed on calibrated equipments using suitable

reference standards. To prove and document the reliability of the methods, validation as per ICH guidelines has been carried out.

The methods proposed are found to be accurate and reproducible, at the same time being simple and rapid. It can be concluded that the proposed methods using HPTLC can be regarded as simple, sensitive, fast, reproducible and cost effective methods for the estimation of Zaltoprofen and Paracetamol in combined dosage form

CONCLUSION

From the previous discussion, it could be concluded that the proposed procedures are simple and do not require sophisticated techniques or instruments. They are also sensitive and selective and could be used for routine analysis of ZLT and PAR in their available dosage form without prior separation. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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