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# A SIMPLE, RAPID, STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC, PARACETAMOL AND TRAMADOL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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**Keywords:**

Simultaneous estimation, Aceclofenac, Paracetamol, Tramadol Hydrochloride, Validated RP - HPLC Method, forced degradation, Stability indicating

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**ABSTRACT:** The drugs Aceclofenac (ACF), Paracetamol (PAR), and Tramadol Hydrochloride (TH) are analgesics, and the combination is used in the symptomatic treatment of moderate to severe pain. The reported HPLC methods for this combination use complicated mobile phases; none is a stability-indicating method. The present study aims to develop and validate a simple stability-indicating RP-HPLC method for simultaneous estimating ACF, PAR, and TH in bulk and tablet dosage form. The chromatographic separation was performed using Neosphere C18 (250 x 4.6 mm, 5µm) with a mobile phase comprising methanol: 0.5% Triethanolamine. 0.5% Triethanolamine was added to methanol to reduce tailing. The flow rate was set to 1.5ml/min and UV detection was carried out at 270 nm with a run time of 4 min. It was found to be linear in the range of 15-40 µg/ml, 39.9- 106.4 µg/ml, and 129.9-346.4 µg/ml with a $R^2$ value of 0.999, 0.999, and 0.995 for TH, ACF and PAR respectively. Statistical analysis proved that the developed method is simple, accurate, precise, reproducible, specific, sensitive and cost-effective. All three drugs were subjected to hydrolytic, acidic, basic, oxidative, thermal, and photodegradation. Degradation of PAR was found in oxidative and acidic conditions. ACF degradation was found in acidic conditions. Tramadol HCl showed degradation in every condition that was employed except hydrolytic condition.

**INTRODUCTION:** Numerous analytical equipment and techniques are available and, among them reverse phase High-Performance Liquid Chromatography (RP-HPLC) stands out due to its versatility, resolution, specificity, accuracy, precision, and cost-effectiveness.

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A stability-indicating assay quantifies an active ingredient in the presence of its degradation products, excipients, or other possible impurities. Forced degradation or stress testing also provides information about the degradation pathways and products that could form during storage [1-4].

Paracetamol (acetaminophen) (PAR), Aceclofenac (ACF) and Tramadol hydrochloride (TH) are important drugs widely used for many clinical purposes. TH is a centrally acting synthetic opioid analgesic and serotonin reuptake inhibitor. ACF is a nonsteroidal anti-inflammatory drug (NSAID) of the class phenylacetic acid derivatives.

It selectively inhibits Cyclooxygenase (COX)-2 enzyme to inhibit the generation of inflammatory mediators and suppresses pro-inflammatory prostaglandins and cytokines. PAR is chemically 4-hydroxy acetanilide. It is a weak inhibitor of peripheral cyclooxygenase, and its analgesic effects may arise from the inhibition of prostanoid synthesis in the CNS. The antipyretic effects of paracetamol are due to inhibiting prostaglandin synthesis [5-8].

The combination of these three drugs is employed for the symptomatic treatment of moderate to severe pain such as muscoskeletal or migraine pain. They provide better analgesia against several types of pain and sources of pain because they have complementary modes of action and target multiple sites [9-10].

After an exhaustive literature search, few HPLC methods are reported of this combination but none of them is stability indicating and also using some complicated mobile phases [10-15]. There are reported stability-indicating assay methods for TH, PAR and ACF individually or in combination with other drugs [16-19]. But, no stability-indicating method has been reported for the simultaneous detection of presently selected drugs. The objective of this research was to develop and validate a simple stability-indicating HPLC assay method for the quantitation of these drugs. The force degradation studies are carried out under hydrolytic, acidic, basic, photolytic, oxidative, and thermal stress conditions. However, as ACF and PAR degradation products are already reported, our main focus was to study the degradation of TH [20-24].

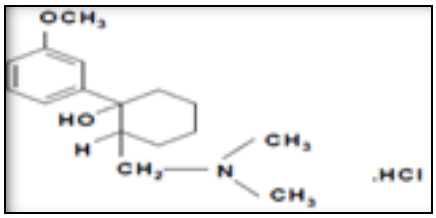


FIG. 1: TH

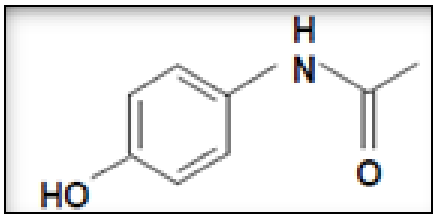


FIG. 2: PAR

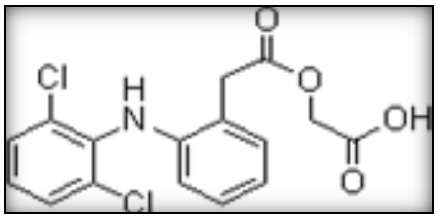


FIG. 3: ACF

## MATERIALS AND METHODS:

**Materials:** TH pure drug was obtained as a gift sample from Cipla Ltd. Maharashtra India, ACF and PAR pure drug was obtained as a gift sample from Cure Pharma Maharashtra India. Zerodoltablet was purchased from a local medical shop. Methanol was of HPLC grade (Merck, Germany). Reagents used for this assay were of analytical grade. The experiment was carried out at Modern college of Pharmacy (for ladies), Moshi Pune, Maharashtra.

**Instruments:** HPLC analysis was performed on Cyberlab™, Model: LC-100 B binary gradient HPLC system, equipped with UV-100, UV-VIS detector. The separation was achieved using a Neosphere C18 (250 x 4.6 mm, 5µm, Hexon laboratories Pvt. Ltd) column and injector was Rheodyne. A Shimadzu Corporation, U.S.A., and Model: AUX-220 balance was used for weighing standards. All the glass wares were rinse thoroughly with double distilled water and dried in hot air oven.Spectral analysis was made on UV-Visible spectrophotometer - Shimadzu Corporation with model no. 1800, Software UV-probe 3.43.

**Selection of Analytical Wavelength:** Standard stock solutions of TH, ACF and PAR were prepared separately with methanol to get final concentration of 200 µg/ml. From standard stock solutions of each drug 20 µg/ml solutions were prepared separately. The UV spectrum of solution was taken and $\lambda_{max}$was found to be 276.4nm, 272.4nm and 248.2nm for ACF, TH and PAR respectively. The iso-absorptive point (isobestic point) for ACF and PAR was found out to be 267.84 nm and thus 270 nm was selected as a wavelength of detection for HPLC analysis.

**Chromatographic System and Conditions:** The composition of the mobile phase is methanol: 0.5% Triethanolamine (v/v). The mobile phase was filtered through a 0.45µm membrane filter and degassed for 10 min. The flow rate was 1.5ml/min, injection volume was 20µL and UV detection was carried out at 270 nm with a run time of 4 min.

**Preparation of Standard and Sample Solutions:** In the marketed formulation (Zerodol PT) TH, ACF and PAR were found to be in the ratio of

1:2.66:8.66. So, the pure drugs were individually weighed in the same ratio and were mixed in geometric proportion using a sieve. The mixture was sieved five times to ensure proper mixing. Powder mixture equivalent to 5mg of TH was weighed and diluted up to 25ml in a volumetric flask with methanol to get a 200 µg/ml stock solution in which ACF and PAR were present as 13.3mg and 43.3mg respectively. The standard stock solution was further diluted with methanol to obtain working standard solutions in a concentration range of 15-40 µg/ml (15, 20, 25, 30, 35, and 40) for TH, 39.9-106.4 µg/ml (39.9, 53.2, 66.6, 79.8, 93.1, and 106.4) of ACF and 129.9-346.4 µg/ml (129.9, 173.2, 216.5, 259.8, 303.1, 346.4) of PAR. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To determine the content of ACF, PAR and TH simultaneously in the pharmaceutical dosage form Zerodol PT, Ten tablets were weighed and powdered and it was made to pass through sieve for about 5 times to obtain a fine powder. The powdered drug equivalent to 5 mg of TH was weighed accurately and transferred into a 25 ml volumetric flask containing little methanol. After sonication for 15 min, the volume was made up to 25ml with methanol. The solution was then filtered through Whatman filter paper. 2 ml of the above solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol. The concentration obtained after the above dilution was 106.4 µg/ml of ACF, 346.4 µg/ml of PAR and 40 µg/ml of TH. The peak areas were measured with the sample's six injections, and each drug's percent content was calculated using a linear regression equation obtained from the calibration curve.

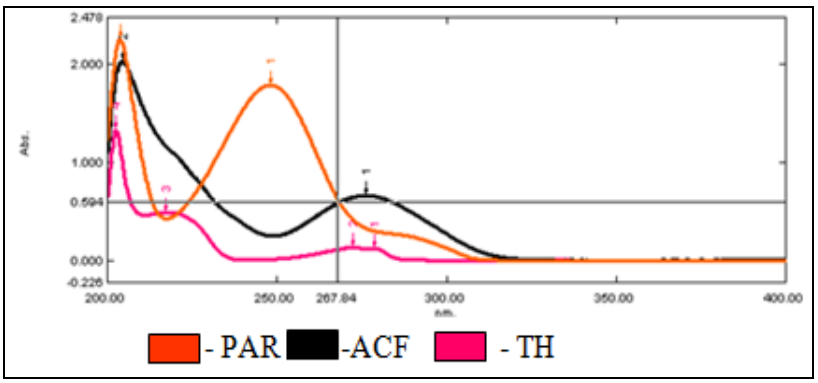


**FIG. 4: OVERLAY OF ACF, PAR, TH**

**Forced Degradation Study:**

**Preparation of Drug Stock Solution:** 1000 µg/ml solution was prepared for all three drugs.

**Hydrolytic Degradation:** 10 ml of drug stock solution was taken in different round bottom flasks and 10 ml of distilled water was added to each. The resultant solutions were refluxed at 85ºC for 2hr. Then the solutions were allowed to cool and 50µg/ml solution was used for analysis.

**Acidic Hydrolysis:** 10 ml of drug stock solution were taken in different round bottom flasks and 10 ml of 1N HCl was added to each. The resultant solutions were refluxed at 85ºC for 2hr. Then the solutions were allowed to cool and pH was adjusted to neutral by using 1N NaOH. A 50µg/ml solution was used for analysis. A similar procedure is followed using 0.1N HCl.

**Basic Hydrolysis:** Acidic hydrolysis followed a similar procedure with 1N NaOH and 0.1 N NaOH, and the pH was adjusted to neutral by using 1N HCl.

**Oxidative Degradation:** Similar procedure as hydrolytic degradation was followed with 6% $H_2O_2$ and 3% $H_2O_2$.

**Thermal Degradation:** All three drugs (in approximate amount) were kept in crucible and exposed to dry heat at 85° C for 2 hrs. A 50µg/ml solution was prepared and analyzed.

**Photodegradation:** All three drugs (in approximate amount) were exposed to U.V light (in U.V chamber) at 2 different wavelengths i.e., 254nm and 365nm. A 10µg/ml solution was prepared and analyzed.

**TLC Analysis of Tramadol HCl:** The standard stock solution of tramadol 100µg/ml and degraded sample were taken and spotted on to pre-coated TLC plates with capillary. Then it was run in selected mobile phase i.e. toluene: ethyl acetate: methanol: glacial acetic acid (3:4:2.8:0.2 v/v/v/v). The spots were visualized in U.V chamber and Rf values were calculated.

**NMR analysis of Tramadol HCl:** After separating degradant from semi-preparative HPLC, the sample was kept in a vacuum desiccator for around 8-10 hrs. to remove moisture from the sample completely. After vacuum application, the solubility of sample was checked in $CDCl_3$ and it was found to be soluble and thus the sample was given for analysis by NMR.

## RESULTS AND DISCUSSION:

**Method Validation:** For the developed RP - HPLC method, methanol was chosen as the mobile phase and 0.5 % Triethanolamine was added to reduce the tailing. As indicated by the Figures shown in **Table 1**, the proposed method is simple, rapid, precise, accurate, and sensitive. It is found that ACF, PAR and TH obey linearity within the concentration ranges39.9-106.4µg/ml, 129.9-346.4µg/ml, and 15-40µg/ml, respectively, with co-relation coefficients as 0.999, 0.996 and 0.999 respectively.

Results show that the method is accurate and precise as the % RSD is less than 2%. The method is reproducible with the % RSD of 1.314 % for ACF, 0.834% for PAR and 1.116% for TH. Sensitivity was determined in terms of LOD and LOQ, which were found to be 3.32 (µg/ml) and 10.07 (µg/ml) for ACF, 1.545(µg/ml) and 4.68(µg/ml) for TH and 8.56(µg/ml) and 25.94(µg/ml) for PAR respectively. The specificity of the HPLC method is ascertained by analyzing standard drugs and the sample. The retention time of ACF, PAR and TH was confirmed by comparing the retention time of the standards, and no interference was observed during the analysis between drugs and excipients in tablet **Fig. 6.**

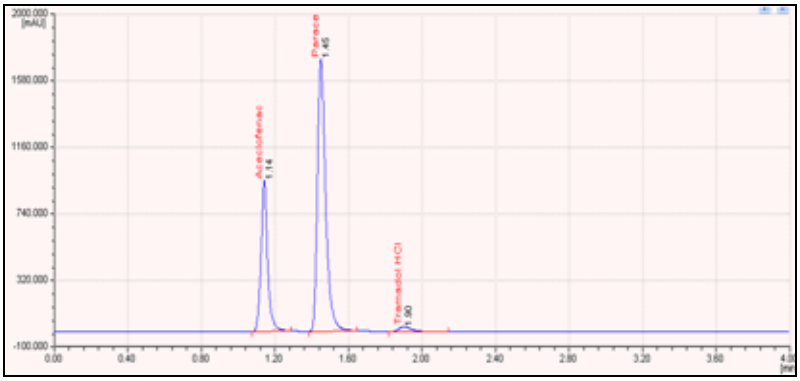


**FIG. 5: HPLC GRAPH OF ACF, PAR AND TH**

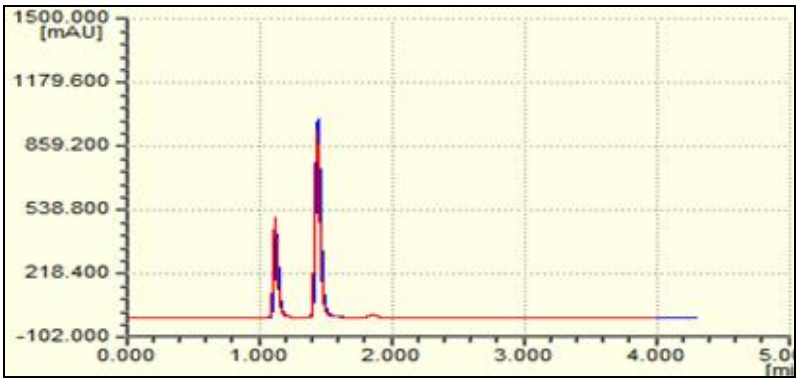


**FIG. 6: GRAPHICAL COMPARISON OF $R_T$ OF TABLET AND STANDARD**

**TABLE 1: SUMMARY OF VALIDATION PARAMETERS**

| Parameters | TH | ACF | PAR |
|---|---|---|---|
| Linearity range (µg/ml) | 15-40 | 39.9-106.4 | 129.9-346.4 |
| Correlation coefficient | 0.996 | 0.999 | 0.999 |

| | | | | |
|---|---|---|---|---|
| Accuracy | (%) Recovery | 99.72 – 100.1 | 97.45 - 98.74 | 99.61 - 100.79 |
| | % RSD | 0.8845 -1.15 | 0.854 - 1.783 | 0.3987 - 1.2314 |
| Precision % RSD intraday | | 1.172 | 0.941 | 0.729 |
| Repeatability % RSD | | 1.116 | 1.314 | 0.834 |
| LOD (µg/ml) | | 1.545 | 3.32 | 8.56 |
| LOQ ( µg/ml) | | 4.68 | 10.07 | 25.94 |

**TABLE 2: ASSAY OF TABLET FORMULATION**

| Drug | Mean of % found* | S.D | RSD | % RSD |
|---|---|---|---|---|
| ACF | 97.26667 | 0.872185 | 0.008967 | 0.896695 |
| PAR | 100.495 | 0.857526 | 0.008533 | 0.853302 |
| TH | 100.5933 | 1.065752 | 0.010595 | 1.059466 |

*Mean of six estimations.

**TABLE 3: SYSTEM SUITABILITY PARAMETERS**

| Parameters | ACF | PAR | TH |
|---|---|---|---|
| Tailing factor | 1.84 | 1.93 | 1.42 |
| Theoretical plates (N) | 4145.8 | 4308.03 | 3352.49 |
| Resolution | 0 | 3.87 | 4.10 |

Degradation of PAR was found only in oxidative and acidic condition but within limit. For ACF 16% degradation was found with 1N HCl. Tramadol HCl showed degradation in every condition that was employed except hydrolytic degradation.

The degradation products of ACF and PAR are already reported so our focus was to study the degradation of Tramadol hydrochloride. Various methods are employed for the analysis of degradation products. The differentiation can be done by comparing retention time of pure Tramadol HCl with the degradant, NMR spectra of pure Tramadol HCl and the degradant and their $R_f$ values. The $R_f$ values of Tramadol HCl, its degradation product in acidic, basic, and photolytic conditions were found to be 0.5, 0.15, 0.1 and 0.43, respectively, calculated using TLC method.

By comparing the NMR spectra of the degradant of Tramadol HCl in basic and photolytic conditions, signals of N-(CH3)2 were absent from the degradant's spectra, and signals for four hydrogen atoms of aromatic ring were absent.

**TABLE 4: FORCED DEGRADATION STUDIES OF TH**

| Condition | Result |
|---|---|
| Hydrolytic | No degradation |
| Acidic | 32.77% degradation with 0.1 N HCl and |
| Basic | 20.17% degradation with 0.1 N NaOH and no degradation with 1 N NaOH |
| Thermal | 42.15% degradation was observed |
| Photolytic | 254nm- 10.92% degradation, 365nm- 12.4 % degradation |
| Oxidative | 8.60% degradation in 3 % $H_2O_2$ |

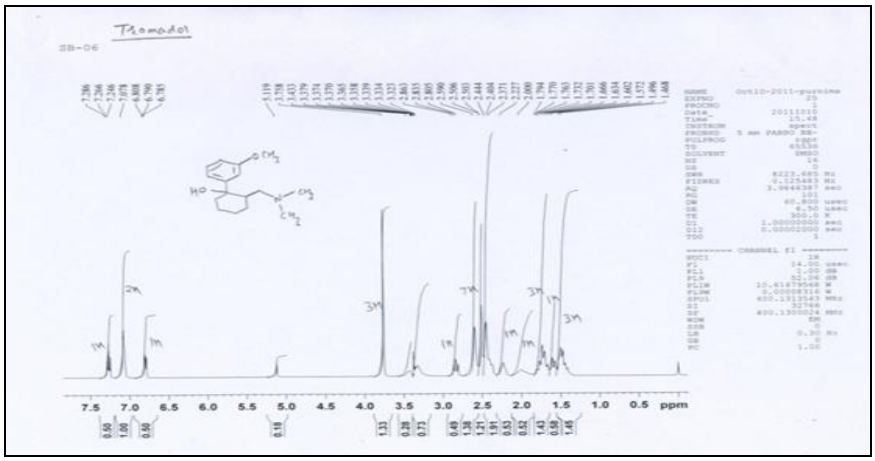


**FIG. 7: NMR SPECTRA OF PURE TH**

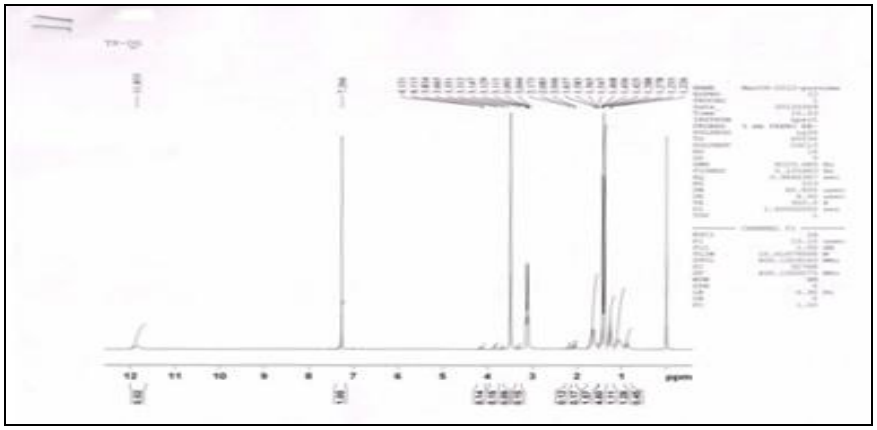

**FIG. 8: NMR SPECTRA OF DEGRADATION PRODUCT OF TH IN BASIC CONDITION**

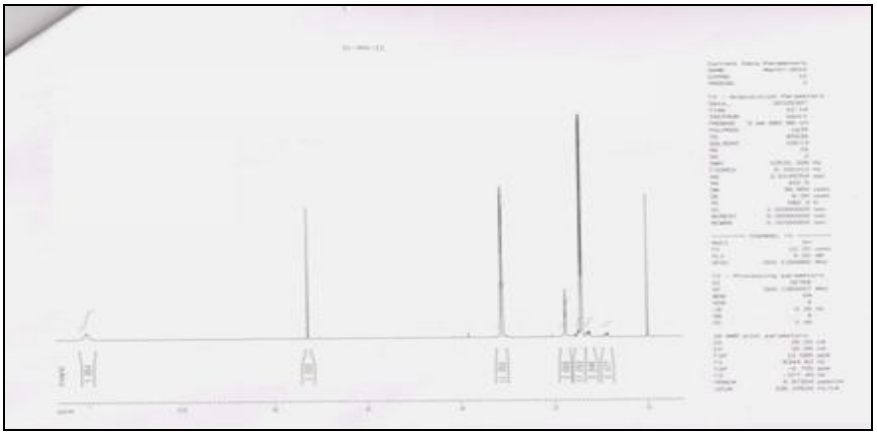

**FIG. 9: NMR SPECTRA OF PHOTODEGRADATION PRODUCT OF TH**

**CONCLUSION:** A rapid and sensitive RP-HPLC method has been developed for the analysis of a ternary drug mixture (Aceclofenac, Paracetamol, and Tramadol Hydrochloride) in bulk and tablet dosage form. The method uses a simple mobile phase, has a rapid run time of 4 minutes that allows analysis of a large number of samples in a given time, and thus has been proven to be cost-effective. The proposed method was validated for the parameters given in ICH guidelines and found to be accurate, precise, sensitive, specific and reproducible.

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**CONFLICTS OF INTEREST:** Nil

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