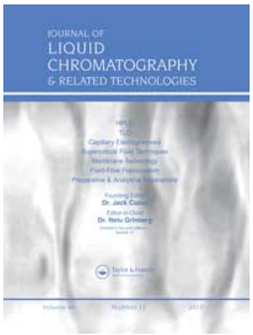


Taylor & Francis
Taylor & Francis Group

Journal of Liquid Chromatography & Related Technologies

ISSN: 1082-6076 (Print) 1520-572X (Online) Journal homepage: http://www.tandfonline.com/loi/ljlc20

# Stability-indicating RP-HPLC method for simultaneous quantitation of tramadol and aceclofenac in presence of their major degradation products: Method development and validation

Md. Gousuddin, Pinaki Sengupta, Bappaditya Chatterjee & Sreemoy Kanti Das

**To cite this article:** Md. Gousuddin, Pinaki Sengupta, Bappaditya Chatterjee & Sreemoy Kanti Das (2017) Stability-indicating RP-HPLC method for simultaneous quantitation of tramadol and aceclofenac in presence of their major degradation products: Method development and validation, Journal of Liquid Chromatography & Related Technologies, 40:17, 887-893, DOI: 10.1080/10826076.2017.1381849

**To link to this article:** https://doi.org/10.1080/10826076.2017.1381849

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
2017, VOL. 40, NO. 17, 887–893
https://doi.org/10.1080/10826076.2017.1381849

Taylor & Francis
Taylor & Francis Group



# Stability-indicating RP-HPLC method for simultaneous quantitation of tramadol and aceclofenac in presence of their major degradation products: Method development and validation

Md. Gousuddin[a], Pinaki Sengupta[b,c], Bappaditya Chatterjee[b], and Sreemoy Kanti Das[a]

[a]Faculty of Pharmacy, Lincoln University College, Selangor, Malaysia; [b]Department of Pharmaceutical Technology, International Islamic University Malaysia, Kuantan, Malaysia; [c]Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gujarat, India

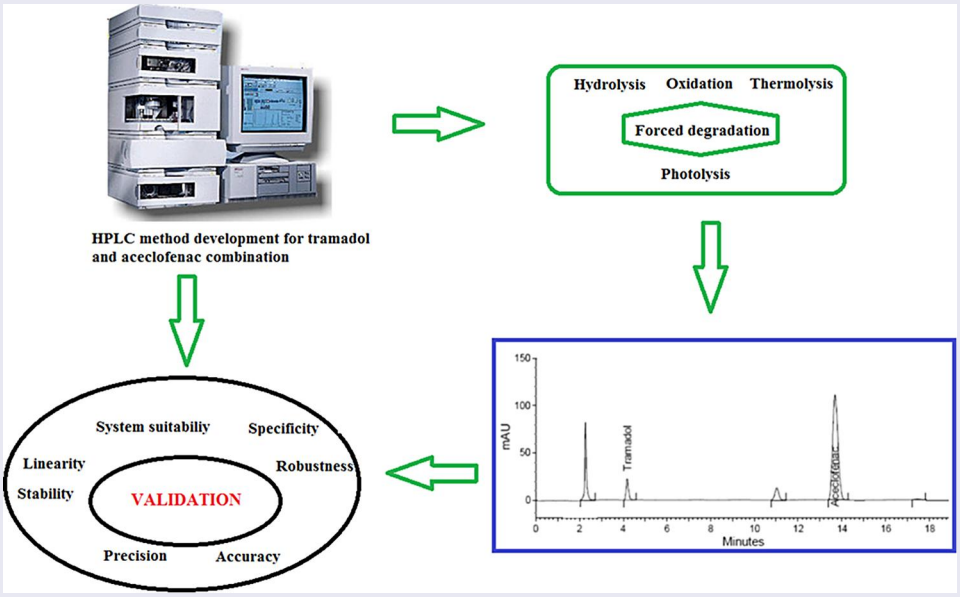
**ABSTRACT**

Primary objective of this study was to develop a stability-indicating reverse-phase high-performance liquid chromatography (HPLC) method for simultaneous quantitation of tramadol and aceclofenac in presence of their degradation products. The drugs were subjected to various International Conference on Harmonization recommended stress conditions, such as acid hydrolysis, alkaline hydrolysis, peroxide oxidation, thermolysis, and photolysis. The major degradation products got well resoluted from the analytes in HPLC analysis with a mobile phase composed of a mixture of 0.01 M ammonium acetate buffer (pH 6.5) and acetonitrile (65:35, v/v) through a Phenomenex Gemini C18 (250 mm × 4.6 mm, 5 µm particle size) column. The method was linear over a range of 15–60 µg/mL for tramadol and 40–160 µg/mL for aceclofenac concentration. The analytes were detected at a wavelength of 270 nm. The method was validated and found to be specific, accurate, precise, stable, and robust for its intended use. The method can be recommended for its future use in routine quality control, accelerated and real-time stability analysis of the formulations containing tramadol and aceclofenac combination.

**KEYWORDS**

Aceclofenac; forced degradation; HPLC; stability-indicating method; tramadol; validation

**GRAPHICAL ABSTRACT**






## Introduction

A stability-indicating assay is an analytical technique to quantify an active ingredient in presence of different other impurities. Stability-indicating methods are able to quantify the drug substance without interference from their degradation products, excipients, or other possible impurities.[1] For release testing of a drug product, the analytical testing procedure should be able to detect and quantify all possible impurities including their degradation products. An analytical assay method used for stability study of pharmaceuticals should be stability indicating unless it has been scientifically justified.[2] The degradation impurities generated from different stress conditions should

**CONTACT** Pinaki Sengupta psg725@gmail.com Opposite Air force Station, Palaj, Gandhinagar, Gujarat 382355, India.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ljlc.

be taken into consideration to evaluate the specificity of the analytical assay method. Analysis of forced degradation samples is performed to demonstrate that degradation product from the drugs and excipients is not interfering with quantification of the drugs.[1,2] Forced degradation or stress studies are conducted in conditions in elevated severity compared to accelerated stability testing conditions for generating the degradation products in a much shorter period of time. Samples generated from stress studies are used to develop the stability-indicating assay method that can be useful to analyze the samples generated from accelerated and long-term stability studies.[2] Several guidelines recommended different stress conditions for this purpose.[3–5] Those guidelines are very general and do not describe the detailed procedure for forced degradation study. The minimum conditions recommended for stress studies should cover acid and base hydrolysis, thermolysis, photolysis, and oxidative degradation.[6–9] Concentration of drug for a stress study has not been mentioned in these regulatory guidances. However, it has been suggested that stress study should be conducted at the concentration of drug likely to be there in final formulation.[10] Reverse-phase (RP) high-performance liquid chromatography (HPLC) coupled with UV detector is widely used analytical technique for separation and quantitation of impurities in pharmaceuticals.[11]

Tramadol, a synthetic analogue of codeine, is a centrally acting analgesic substance.[12,13] Metabolism of tramadol by cytochrome P450 in liver generates *O*-desmethyltramadol, which actually acts as an analgesic metabolite.[14–17] Tramadol is a widely used opioid analgesic agent nowadays. It is mostly available as hydrochloride salt and is marketed as injectables and oral formulations. Aceclofenac is also an analgesic agent with good tolerability. It is particularly useful in treating rheumatic conditions and soft tissue injury.[18,19] Aceclofenac exerts its anti-inflammatory potential by inhibiting cyclooxygenase enzyme and prostaglandin biosynthesis.[20,21] Combination of tablet formulation containing tramadol hydrochloride and aceclofenac is available in the market for limited period treatment of medium to high painful events. The combination got better analgesic efficiency compared to individual drugs.

Objective of this research was to develop and validate a stability-indicating HPLC assay method for quantitation of tramadol and aceclofenac even in the presence of their major degradation products. There are few previously reported stability-indicating assay method for tramadol[22–24] and aceclofenac[25–27] individually or in combination with other drugs. But, no stability-indicating method has been reported for simultaneous detection of tramadol and aceclofenac. Even, there is no other type of analytical method is available for their simultaneous quantitation. To the best of our knowledge, this method is a novel analytical technique to analyze tramadol and aceclofenac in their combination as well as in presence of their possible impurities.

## Experimental

### Materials

Tramadol hydrochloride and aceclofenac were obtained from Intas Pharmaceutical Ltd, India. The HIFENAC-TL tablet (Intas Pharmaceuticals Ltd) that contains 37.5 mg of tramadol and 100 mg of aceclofenac was purchased from pharmacy shop. HPLC-grade acetonitrile, methanol, and water were purchased from Spectrochem, India. Analytical-grade hydrochloric acid, sodium hydroxide, acetic acid, and hydrogen peroxide (30%, v/v) were purchased from Ranbaxy Fine Chemicals, India. Triethyl amine and ammonium acetate were purchased from Merck KGaA, Germany.

### Instrumentation

The analytical instrument used for the development and validation of this stability-indicating method was Shimadzu HPLC system (Kyoto, Japan) equipped with LC-20AT pump and UV–Visible detector SPD-20A with Spinchrom software.

### Preparation of mobile phase

The mobile phase consisted of a mixture of 0.01 M ammonium acetate buffer of pH 6.5 and acetonitrile at a ratio of 65:35 (v/v). To prepare the buffer solution, 0.77 g of ammonium acetate was dissolved in 1000 mL of HPLC-grade water. pH was adjusted with triethylamine and glacial acetic acid. Triethylamine was added as an additive, whereas glacial acetic acid as a pH adjuster. Mobile phase was filtered through 0.45-µm nylon membrane filter (Millipore Pvt Ltd, India) and degassed using an ultrasonic bath (Spincotech Pvt Ltd, India).

### Preparation of diluent

Acetonitrile and water were mixed in a ratio of 1:1 (v/v) and used as diluent for the entire analysis.

### Preparation of standard solution

A primary standard solution of tramadol (37.5 µg/mL) and aceclofenac (100 µg/mL) combination was prepared by dissolving tramadol hydrochloride powder equivalent to 18.75 mg of tramadol and 50 mg of aceclofenac in 50 mL of diluent. Finally, 5 mL of the primary solution was diluted to 50 mL with diluent.

### Preparation of test solution

Twenty HIFENAC-TL tablets were weighed and the average tablet weight was determined. Tablets were crushed in mortar with pestle and the powder equivalent to five average tablet weight was taken. To this, 50 mL of methanol and 300 mL of diluent were added and subjected to sonication for 20 min. The solution was allowed to return to ambient conditions and diluted to 500 mL with diluent. The solution was then filtered through 0.45-µm nylon syringe filter. An amount of 5 mL of the filtered solution was diluted to 50 mL with diluent. The concentration of tramadol and aceclofenac in the final test solution was 37.5 and 100 µg/mL, respectively.

### Chromatographic conditions

Cromatographic conditions were selected based on the chemical property and solubility of tramadol and aceclofenac.

Method was finalized after several trials using the degraded sample of each component as analytes of interest. Tramadol and aceclofenac were dissolved in polar solvents for chromatographically separate them using a nonpolar stationary phase. Column was selected based on the peak shape, theoretical plate count, retention of analytes, and resolution between tramadol, aceclofenac, and their degradant peaks.

### Forced degradation

To suitably develop the HPLC method to quantify tramadol and aceclofenac in presence of their possible degradants, the combined test solutions were subjected to various deliberate stress conditions to generate their degradation products. Different regulatory guidelines (ICH Q1A, Q2A, Q2B, Q3B) indicate the necessity of developing and validating stability-indicating assay methods for the drugs. These guidelines suggested to select the nature of stress conditions depending on the type of individual analytes involved.[28–32]

The scientific and regulatory considerations for forced degradation conditions of the drugs were based on the above ICH guidelines in addition to different previously reported articles.[33–36] Following those recommendations, in this study, the stress conditions, amount of stress substances, and time of stresses were selected based on development trials that were able to generate the degradation products of the drugs. Degradation samples were prepared by dissolving the powdered tablet in a 500-mL round-bottom flask. Samples were then subjected to hydrolytic (acidic, alkaline), oxidative, thermal, and photolytic stress conditions. After exposing to degradation conditions, stress-induced sample solutions were allowed to return to room temperature and diluted with mobile phase to obtain 37.5 µg/mL of tramadol and 100 µg/mL of aceclofenac concentration.

### Hydrolytic degradation

Hydrolysis is one among the most common degradation procedures covering entire pH range. Hydrolysis causes breakdown of compounds by reaction with water. Acidic and alkaline hydrolysis involve lysis of ionizable functional groups of the compounds. In this study, acidic hydrolytic degradation was performed by refluxing the drug solution in 50 mL of 0.1 N HCl for 30 min. The mixture was then neutralized with 0.1 N NaOH solution. Alkaline hydrolytic degradation was performed by refluxing the sample solution in 0.05 N NaOH (50 mL) for 15 min. The mixture was then neutralized with 0.05 N HCl solution.

### Oxidative degradation

Despite the presence of wide varieties of oxidizing agents, hydrogen peroxide is widely used for forced degradation of drugs by oxidation. Oxidative degradation of chemical compounds involves an electron transfer procedure to generate reactive ions. In this study, oxidative degradation of tramadol and aceclofenac was performed by refluxing the drug solution in 5% v/v hydrogen peroxide (50 mL) for 3 h.

### Thermal degradation

Heat-induced degradation was performed at higher temperatures for a shorter period of time by exposing the powdered drug in solid state to 80°C in an oven for 72 h.

### Photolytic degradation

Photolytic degradation was performed to generate primary degradants of the drug substances by exposure to sunlight. Light-induced oxidation of the compounds mediates by a free radical mechanism. In this research, photolytic degradation was performed after exposure of the sample in sunlight for 72 h.

## Method validation

### System suitability

A system suitability test (SST) for the HPLC system was performed prior to each validation test. Six replicate injections of standard preparations were injected and the SST parameters such as asymmetry factor, theoretical plate, and %RSD of the peak area of six runs were determined. Acceptance criteria were set to confirm that asymmetry factor should be less than 1.5, theoretical plate should be more than 2000 for tramadol and 5600 for aceclofenac, and %RSD of the six standard peak area should be less than 2.0.

### Specificity

The specificity of the method was determined by evaluating the interference of degradation impurities with the analytes and determining peak purity of tramadol and aceclofenac in forced degraded samples.

### Linearity

Seven different combined calibration solutions containing 15, 22.5, 30, 37.5, 45, 52.5, and 60 µg/mL for tramadol and 40, 60, 80, 100, 120, 140, 160 µg/mL for aceclofenac were prepared. A seven-point calibration curve was constructed and evaluated for their linearity in the above ranges. In linearity study, acceptance criteria for correlation coefficient of the linearity curve were set to be $\geq$0.999.

### Limit of detection and limit of quantitation

Limit of detection (LOD) and limit of quantitation (LOQ) were evaluated by serial dilutions of tramadol and aceclofenac stock solution in lower concentration ranges around their lower LOQs. LOD and LOQ were determined following the signal to noise ratio of 3:1 and 10:1, respectively.

### Accuracy

Standard concentrations of tramadol and aceclofenac were 37.5 and 100 µg/mL, respectively. Accuracy in assay of the method was determined in triplicate at three different concentration levels of 50, 100, and 150% of standard concentration. Concentration levels of tramadol were 18.75, 37.5, and 56.25 µg/mL, whereas for aceclofenac were 50, 100, and 150 µg/mL.

### Precision

Intra-day and inter-day precision study were performed by injecting the standard solution of tramadol (37.5 µg/mL) and aceclofenac (100 µg/mL) in six sets in two different days.

### Solution stability

Stability of the analytes in solution state was determined by keeping the standard solutions at 2–5°C and room

temperature for 48 h. Standard samples were analyzed for determining its accuracy at the initial time and after 12, 24, 36 and 48 h.

### Robustness

The robustness of the developed method was checked by injecting standard solution after minor alteration in flow rate (0.9–1.1 mL/min), mobile-phase composition (acetonitrile content 33–37%), buffer pH (6.3–6.7), and column (different columns of same specification). Theoretical plate count, asymmetry factor, and assay (%) of the injected standard were calculated in different deliberately altered conditions.

## Results and discussion

### Development and optimization of the HPLC method

Satisfactory chromatographic separation between the analytes and their degradants was achieved with Phenomenex Gemini C18 (250 mm × 4.6 mm, 5 µm particle size) reverse-phase column. In aqueous part of the mobile phase, a buffer was included to ensure better control of pH in subsequent analysis. Initially, disodium hydrogen orthophosphate and ammonium acetate were used, however, best peak shape was observed with 0.01 M ammonium acetate buffer after adding 3 mL triethylamine in 1000 mL of buffer and adjusting the pH at 6.5 with glacial acetic acid. Triethylamine as a mobile phase additive showed to lower the peak asymmetry and tailing as well as improved peak shape and resolution. Acetonitrile was found as most suitable organic phase to reduce long retention time and produce good peak shape. The optimized mobile phase consisted of a mixture of 0.01 M ammonium acetate buffer (pH 6.5) and acetonitrile (65:35, v/v). Above mobile phase ratio provided good resolution between tramadol, aceclofenac, and their degradation products. Flow rate of the mobile phase was set at 1.0 mL/min and injection volume was 20 µL. Analytes were detected at a wavelength of 270 nm. Retention times of tramadol and aceclofenac were found to be 4.2 and 13.8 min, respectively. Total chromatographic run time was 19 min. Figure 1 represents the chromatogram of a standard preparation.

### Forced degradation

#### Hydrolytic degradation

A degradation peak of about 30.5% of total chromatographic peak area was observed in acidic hydrolysis. In alkali degradation, aceclofenac was found to be highly unstable and peak of aceclofenac got disappeared. The major degradation peak due to hydrolytic degradation was observed at the retention time of 11.3 min (Figure 2).

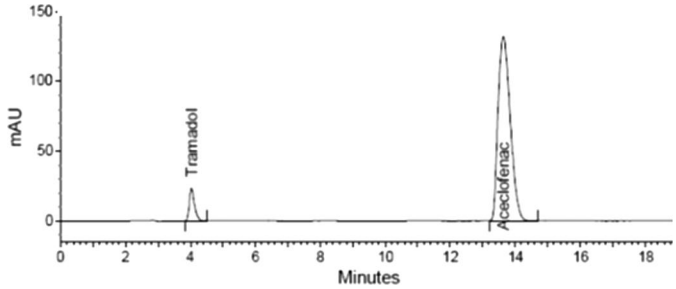



Figure 1. Representative chromatogram of standard solution.

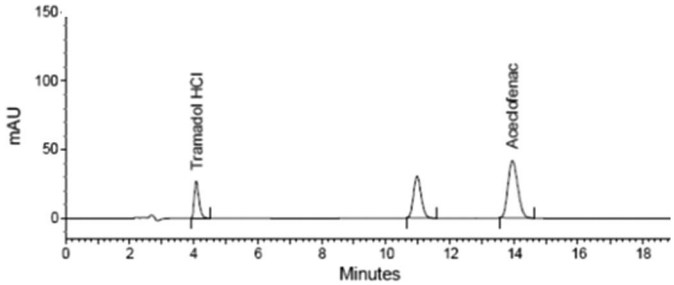



Figure 2. Representative chromatogram of hydrolytically degraded sample.

#### Oxidative degradation

In oxidative degradation, a total of 7% degradation was observed for both the drugs. Retention time of the two major degradation products was about 2.5 and 11.3 min (Figure 3).

#### Thermal degradation

Drugs were found relatively stable under thermal degradation condition. Peak area of the degradation product at RT 11.3 min was only 3.0% (Figure 4).

#### Photolytic degradation

There was very little degradation observed in photolytic condition. Drugs were found stable compared to the exposure in other stress condition (Figure 5).

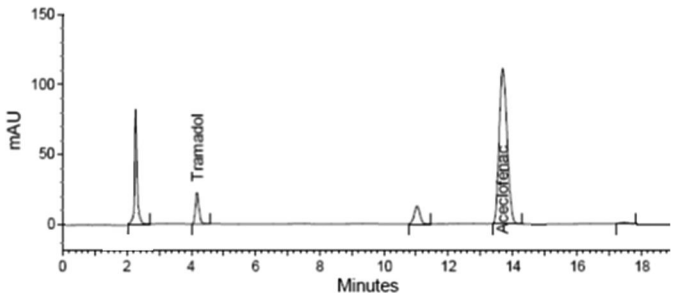



Figure 3. Representative chromatogram of peroxide degraded sample.

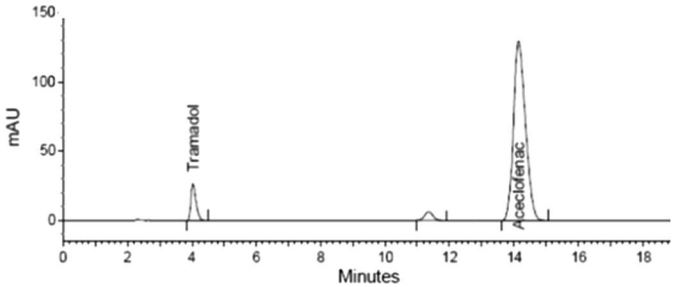



Figure 4. Representative chromatogram of heat degraded sample.

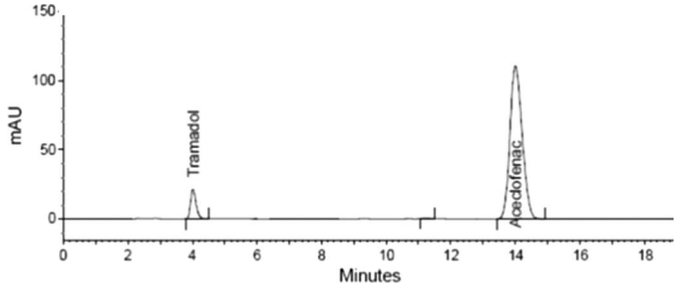



**Figure 5.** Representative chromatogram of photo degraded sample.

**Table 1.** Accuracy data of the analytes.

| Analyte | Level (%) | Amount added (µg/mL) | Amount found (µg/mL) | Accuracy (%) | RSD (%) |
|---|---|---|---|---|---|
| Tramadol | 50 | 19.87 | 19.47 | 97.99 | 0.48 |
| | 100 | 37.87 | 36.93 | 97.54 | 0.67 |
| | 150 | 57.77 | 57.73 | 99.94 | 1.52 |
| Aceclofenac | 50 | 50.33 | 48.73 | 96.83 | 0.91 |
| | 100 | 99.24 | 97.90 | 98.65 | 0.24 |
| | 150 | 151.07 | 151.77 | 100.47 | 1.51 |

**Table 2.** Intra-day and inter-day precision data of the analytes.

| Run | Tramadol | | Aceclofenac | |
|---|---|---|---|---|
| | Mean assay (%) | | Mean assay (%) | |
| | Day 1 | Day 2 | Day 1 | Day 2 |
| 1 | 101.3 | 97.1 | 96.4 | 97.8 |
| 2 | 98 | 98.6 | 96.1 | 98 |
| 3 | 98.4 | 97.2 | 97.4 | 96.1 |
| 4 | 99.3 | 97.8 | 96 | 98.8 |
| 5 | 97.2 | 99.1 | 96.6 | 97.1 |
| 6 | 98.7 | 97.8 | 97.3 | 97.3 |
| Intra-day precision ($n = 6$) | | | | |
| Mean | 98.8 | 97.9 | 96.6 | 97.5 |
| SD | 1.40 | 0.78 | 0.60 | 0.92 |
| RSD (%) | 1.42 | 0.80 | 0.62 | 0.94 |
| Inter-day precision ($n = 12$) | | | | |
| Mean | 98.4 | | 97.1 | |
| SD | 1.18 | | 0.87 | |
| RSD (%) | 1.20 | | 0.89 | |

**Table 3.** Solution stability data of the analytes.

| Time (h) | Assay (%, at 2–5°C) | | Assay (%, at ambient temp.) | |
|---|---|---|---|---|
| | Tramadol | Aceclofenac | Tramadol | Aceclofenac |
| Initial | 97.8 | 100.6 | 101.5 | 97.9 |
| 12 | 98.3 | 102.7 | 98.4 | 96.8 |
| 24 | 96.8 | 99.2 | 97.6 | 100.3 |
| 36 | 100.5 | 97.9 | 100.5 | 101.8 |
| 48 | 98.5 | 97.7 | 99.3 | 99.7 |

## Method validation

### System suitability

System suitability was evaluated before injecting the samples in each individual validation study. SST parameters met the set acceptance criteria for asymmetry factor (less than 1.5), theoretical plate (more than 2000 for tramadol and 5600 for aceclofenac) and %RSD of peak area of six standard injections (less than 2.0%) in all of the validation studies.

### Specificity

Peak purity of tramadol and aceclofenac was found satisfactory in the injected stress-induced degradation samples. No interference of degradants was observed at the retention time of tramadol and aceclofenac. Degradation peaks in the forced degradation sample were well resolved from the analyte peaks.

### Linearity

Response of the analytes was linear over the concentration range of 15–60 µg/mL for tramadol 40–160 µg/mL for aceclofenac. Correlation coefficient of the linearity curve for both the analytes was 0.9999.

### LOD and LOQ

Limit of quantitation value for tramadol and aceclofenac was 0.5 and 0.4 µg/mL, respectively. LOD was found to be 0.15 µg/mL for both the analytes. HPLC chromatograms of LOD and LOQ study showed to have well identifiable peaks for tramadol and aceclofenac.

### Accuracy

Range of mean accuracy in the assay for tramadol and aceclofenac was 97.54–99.94% and 96.83–100.47%, respectively (Table 1). Therefore, result supports the accuracy of the method.

### Precision

Intra-day and inter-day precision data for tramadol and aceclofenac are summarized in Table 2. RSD values in intra-day precision study (six replicates) for tramadol were 1.42 (day 1) and 0.80% (day 2). RSD values in intra-day precision study (six replicates) for aceclofenac were 0.62 (day 1) and 0.94% (day 2). However, RSD values in inter-day precision study (12 replicates including day 1 and day 2) for tramadol and aceclofenac were 1.20 and 0.89%, respectively.

### Solution stability

Table 3 shows the results of solution stability analysis at various time intervals. Analytes were found to be stable up to 48 h at room temperature and 2–5°C in solution state.

**Table 4.** Data of robustness study.

| Conditions | Tramadol | | | Aceclofenac | | |
|---|---|---|---|---|---|---|
| | Theoretical plate count | Assymetry factor | Assay (%) | Theoretical plate count | Assymetry factor | Assay (%) |
| Flow 0.9 mL/min | 3348 | 1.24 | 97.7 | 7206 | 1.29 | 101.4 |
| Flow 1.1 mL/min | 3516 | 1.28 | 101.5 | 7411 | 1.33 | 102.6 |
| Buffer–ACN (63:37, v/v) | 3216 | 1.37 | 98.0 | 6990 | 1.25 | 98.2 |
| Buffer–ACN (67:33, v/v) | 3310 | 1.31 | 97.5 | 7174 | 1.27 | 100.6 |
| Buffer pH 6.7 | 3256 | 1.27 | 99.3 | 7235 | 1.33 | 99.1 |
| Buffer pH 6.3 | 3528 | 1.33 | 101.6 | 7001 | 1.30 | 97.5 |
| Column change | 3191 | 1.26 | 97.7 | 7392 | 1.27 | 98.8 |

### *Robustness*

Observations in robustness study are summarized in Table 4. Results indicate that percentage assay value of the injected solution was not affected due to slight variation of different chromatographic conditions. System suitability parameters were also found satisfactory in robustness study.

## Discussion

In this study, a simple stability-indicating RP-HPLC method has been developed and validated for determining tramadol and aceclofenac in tablet dosage form. The combined drugs were exposed to different ICH recommended stress conditions. Outcome of the tested parameters for validation study confirmed that the developed method is a linear, accurate, precise, stable, reproducible, and stability-indicating quantitative analytical technique for quantification of tramadol and aceclofenac in combination. Forced degradation of the two drugs was performed under all suggested conditions and the degradation products got well resolved in a single isocratic run in this method. The method was found to be specific for the two analytes, even in presence of their possible major degradation products. Acceptable values of precision and accuracy have been observed at all tested levels. The method was also found robust when tested in different varied chromatographic conditions. As commercial tablet formulation is already in market and effectiveness of the combination is already established, there is all possibility to have more research on development of new formulations for the combination of tramadol and aceclofenac. This HPLC method can immensely help its manufacturers and researchers to analyze the stability samples in their research and routine quality control procedure. The method is very simple and sensitive with a lower LOQ of 15 µg/mL for tramadol and 40 µg/mL for aceclofenac. Because of its shorter run time, the method will reduce total analysis time and thus minimize the incurred cost of sample analysis compared to their individual methods. Therefore, this method can be claimed as more economical and can be used for stability testing as well as routine quality control analysis of tramadol and aceclofenac combination in bulk drug and pharmaceutical formulations.

## Conclusion

There was no reported stability indicating assay method for simultaneous quantitation of tramadol and aceclofenac in either bulk drug or in any pharmaceutical dosage form. Therefore, the method developed in present investigation is a novel of its kind. This validated method will be useful to the researcher and pharmaceutical manufacturer for routine quality control and stability sample analysis of the combined formulation of tramadol and aceclofenac. The method will offer some definite economic benefit over their individual existing analytical methods due to its ability to quantitate the two analytes simultaneously in a single chromatographic run and reduction of consumption of solvents, chemicals, time, and manpower.



## Funding

Authors are grateful to Lincoln University College Malaysia and International Islamic University Malaysia for providing the necessary instruments and financial supports to perform this research.

## References

[1] Sawant, S.; Ghante, M. A Validated Stability Indicating RP-HPLC Method for Simvastatin, Identification and Characterization of Forced Degradation Products of Simvastatin using LC-ESI-MS/MS. *Adv. Anal. Chem.* **2015**, *5*, 8–16.
[2] Blessy, M.; Patel, R. D.; Prajapati, P. N.; Agrawal, Y. K. Development of Forced Degradation and Stability Indicating Studies of Drugs—A Review. *J. Pharm. Anal.* **2014**, *4*, 159–165.
[3] ICH guidelines. **2003**, November, 25.
[4] Baertschi, S. W.; Alsante, K. M.; Tønnesen, H. H. A Critical Assessment of the ICH Guideline on Photostability Testing of New Drug Substances and Products (Q1B): Recommendation for Revision. *J. Pharm. Sci.* **2010**, *99*, 2934–2940.
[5] EMA-stability. *Regul. EMA*, **2003**, December, 1–18.
[6] FDA. Stability Guidelines. *Cent. Drug Eval. Res.* **1998**, *22*, 1–30.
[7] FDA. Guidance for Industry. *Cent. Drug Eval. Res.* **2015**, No. July, 1–15.
[8] Chakole, R. D.; Charde, M. S.; Kumar, J.; Welankiwar, A. S. Review: Development of Forced Degradation Studies of Drugs. *Int. J. Adv. Pharm.* **2013**, *2*, 34–39.
[9] Srinivasulu, K.; Naidu, M. N.; Rajasekhar, K.; Veerender, M.; Suryanarayana, M. V. Development and Validation of a Stability Indicating LC Method for the Assay and Related Substances Determination of a Proteasome Inhibitor Bortezomib. *Chromatogr. Res. Int.* **2012**, *2012*, 1–13.
[10] Bakshi, M.; Singh, S. Development of Validated Stability-Indicating Assay Methods—Critical Review. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1011–1040.
[11] Qiu, F.; Norwood, D. L. Identification of Pharmaceutical Impurities. *J. Liq. Chromatogr. Relat. Technol.* **2007**, *30*, 877–935.
[12] Keskinbora, K.; Aydinli, I. Atipik opioid analjezik: tramadol. *Agri.* **2006**, *18*, 5–19.
[13] Glavanović, S.; Glavanović, M.; Tomišić, V. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2016**, *157*, 258–264.
[14] Subrahmanyam, V.; Renwick, A. B.; Walters, D. G.; Young, P. J.; Price, R. J.; Tonelli, A. P.; Lake, B. G. Identification of Cytochrome P-450 Isoforms Responsible for Cis-Tramadol Metabolism in Human Liver Microsomes. *Drug Metab. Dispos.* **2001**, *29*, 1146–1155.
[15] Perez, T. E.; Mealey, K. L.; Grubb, T. L.; Greene, S. A.; Court, M. H. Tramadol Metabolism to O-desmethyl Tramadol (M1) and N-desmethyl Tramadol (M2) by Dog Liver Microsomes: Species Comparison and Identification of Responsible Canine Cytochrome P-450s (CYPs). *Drug Metab. Dispos.* **2016**, *44*, 1963–1972.
[16] Overholser, B. R.; Foster, D. R. Opioid Pharmacokinetic Drug-Drug Interactions. *Am. J. Manag. Care* **2011**, *17*, S276–S287.
[17] Smith, H. S. Opioid Metabolism. *Mayo Clin. Proc.* **2009**, *84*, 613–624.
[18] Ravisankar, P.; Rao, G. D.; Saibabu, S.; Devadasu, C.; Babu, P. S.; Sricharitha, P.; Kumar, K. V. S. S. Development and Validation of a Reverse Phase HPLC Method for the Determination of Aceclofenac in Bulk and Pharmaceutical Dosage Forms. *Int. J. Res. Pharm. Chem.* **2013**, *3*, 483–494.
[19] Chandra, P.; Rathore, A. S.; Lohidasan, S.; Mahadik, K. R. Application of HPLC for the Simultaneous Determination of Aceclofenac, Paracetamol and Tramadol Hydrochloride in Pharmaceutical Dosage Form. *Sci. Pharm.* **2012**, *80*, 337–351.
[20] Pavithra, P.; Dhanraj, M.; Sekhar, P. Analgesic Effectiveness of Ibuprofen and Aceclofenac in the Management of Acute Pulpitis – A Randomized Double Blind Trial. *Int. J. Pharm. Sci. Rev. Res.* **2015**, *35*, 70–74.
[21] Cho, H.; Walker, A.; Williams, J.; Hasty, K. A.; Shuang, Y. Study of Osteoarthritis Treatment with Anti-Inflammatory Drugs:

Cyclooxygenase-2 Inhibitor and Steroids. *Biomed. Res. Int.* **2015**, *2015*, 1–10.
[22] Vinay, K. B.; Revanasiddappa, H. D.; Xavier, C. M.; Ramesh, P. J.; Raghu, M. S. Stability Indicating UPLC Method for the Determination of Tramadol Hydrochloride: Application to Pharmaceutical Analysis. *Chromatogr. Res. Int.* **2012**, *2012*, 1–9.
[23] Kamble, R. M.; Singh, S. G. Stability-Indicating RP-HPLC Method for Analysis of Paracetamol and Tramadol in a Pharmaceutical Dosage Form. *E-J. Chem.* **2012**, *9*, 1347–1356.
[24] Karunakaran, K.; Navaneethan, G.; Elango, K. P. Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Determination of Paracetamol, Tramadol HCl and Domperidone in a Combined Dosage Form. *Trop. J. Pharm. Res.* **2012**, *11*, 99–106.
[25] Azhlwar, S.; Ravi, T. K. Stability Indicating HPLC Method for Simultaneous Determination of Drotaverine and Aceclofenac. *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 245–250.
[26] Hossain, M. F.; Bhadra, S.; Kumar, U.; Rouf, A. S. S. The ICH Guidance in Practice: Stress Degradation Studies on Aceclofenac and Development of a Validated Stability-Indicating Reversed-Phase HPLC Assay in Tablet Dosage Form. *Der. Pharm. Chem.* **2013**, *5*, 131–146.
[27] Bhinge, J. R.; Kumar, R. V; Sinha, V. R. A Simple and Sensitive Stability Indicating RP-HPLC Assay Method for the Determination of Aceclofenac. *J. Chromatogr. Sci.* **2008**, *46*, 440–444.
[28] ICH. *Int. Conf. Harmon.* **2003**, No. February, 24.
[29] International Conference on Harmonisation B. *ICH Harmon. Tripart. Guidel.* **2006**, No. June, 12.
[30] ICH. *Int. Conf. Harmon. Tech. Requir. Regist. Tripart. Guidel.* **1996**, No. November, 13.
[31] U.S. Food and Drug Administration/Center for Biologics Evaluation and Research. *Food Drug Adm.* **1995**, No. March, 1–9.
[32] ICH. *Int. Conf. Harmon.* **2005**, *1994*, 17.
[33] Bakshi, M.; Singh, S. Development of Validated Stability-Indicating Assay Methods—Critical Review. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1011–1040.
[34] Rawat, T.; Pandey, I. P. Forced Degradation Studies for Drug Substances and Drug Products – Scientific and Regulatory Considerations. *J. Pharm. Sci. Res.* **2015**, *7*, 238–241.
[35] Sehrawat, R.; Maithani, M.; Singh, R. Regulatory Aspects in Development of Stability-Indicating Methods: A Review. *Chromatographia* **2010**, *72*, 1–6.
[36] Ngwa, G. Forced Degradation as an Integral Part of HPLC Stability-Indicating Method Development. *Drug Deliv. Technol.* **2010**, *5*, 56–59.