Stability Indicating Validated HPLC Method for the Determination of Aceclofenac and Misoprostol in Bulk and Pharmaceutical Formulation

Syed Rafi*, Kantipudi Rambabu

Department of chemistry, RVR & JC College of Engineering, Chowdavaram, Guntur-522019, Andhra Pradesh, India

Article History:
Received on: 07 Nov 2020
Revised on: 11 Dec 2020
Accepted on: 15 Dec 2020

Keywords:
Aceclofenac, Misoprostol, Validation, HPLC, Stress studies

ABSTRACT

For the simultaneous evaluation of Aceclofenac and Misoprostol using RP-HPLC, an accurate, rapid, economical, and straightforward, reliable assay technique was developed and demonstrated. The proposed method achieved effective chromatographic separation by using an inertsil ODS column (150mmx4.6mm, 3.5), acetonitrile, and 0.1 percent orthophosphoric acid (OPA) (50:50 v/v) as a mobile phase with a flow rate of 1 ml/min and a wavelength of 227 nm. The retention time (Rt) of Aceclofenac was 3.189 minutes and Misoprostol was 6.966 minutes. Chromatography was performed isocratically at room temperature with a run time of around 10 minutes. The suitability parameters of the system were investigated by multiplying the quality six times, and the results were well within acceptable limits. The linearity analysis was conducted at 10 percent to 150 percent stages, with a regression coefficient of 0.999. Aceclofenac and Misoprostol had LOD and LOQ values of 0.063 µg/ml, 0.063 g/ml, and 0.208 µg/ml and 0.208 g/ml, respectively. The drug was recovered at a rate of 98-102 percent, which means that the recovery is within reasonable limits. The validation results were satisfactory, and the approach was found to be suitable for bulk and formulation analysis. As a result, it was obvious that the proposed approach was ideal for routine pharmaceutical preparation review and quality control. Validation results were very close to the appropriate maximum. RSD values of less than 2.0 percent indicate that this approach is accurate and precise. The above method was used to perform a retail formulation assay, which showed that 100.24 percent of the formulation was present. Degradation stress conditions in acidic, alkaline, peroxide, and thermal media were investigated. Under ideal conditions, the established method provided efficient, precise, and accurate results. According to ICH guidelines, the approach was justified.

INTRODUCTION

Aceclofenac is a nonsteroidal anti-inflammatory drug (Machado et al., 2017) (NSAID) analog of diclofenac. It is used for the relief of pain and inflammation (Mantovani et al., 2008) in rheumatoid arthritis (Smolen et al., 2016), osteoarthritis (Glyn-Jones et al., 2015) and ankylosing spondylitis (Smith, 2015). Aceclofenac is not approved for infants or people with porphyria (Stein et al., 2017; Cherem et al., 2005). It is also not recommended for mothers who are breastfeeding (Kramer and
Kakuma, 2012). It should be avoided in a pregnant woman near term due to the possibility of premature ductus arteriosus closure (Bouayad et al., 2001), which can lead to foetal hydrops (Isaacs, 2008).

Misoprostol is a synthetic prostaglandin (Ricciotti and FitzGerald, 2011) drug that is used to prevent and treat stomach ulcers, induce labour, induce abortion, and treat postpartum bleeding caused by uterine incompetence. When used to avoid gastric ulcers in people taking NSAIDs, misoprostol is taken by mouth. It may be used alone or in combination with mifepristone or methotrexate to induce abortions (Kulier et al., 2004). Abortion efficacy varies between 66 and 90 percent on its own (Bryant et al., 2014; Raymond et al., 2019). It can also be used rectally for postpartum bleeding (Blum et al., 2007). Diarrhea and abdominal pain are two common side effects. It’s a pregnancy category X drug, which means it’s known to cause harm to the foetus if taken during pregnancy. Uterine rupture (Murphy, 2006) can happen in rare cases. It’s a prostaglandin analogue (Winkler and Fautsch, 2014), more precisely a synthetic prostaglandin E1 (PGE1). The following Figure 1 shows the chemical structures of Aceclofenac and Misoprostol.

Figure 1: Chemical structures of (A) Aceclofenac and (B) Misoprostol

**MATERIALS AND METHODS**

**Chemicals**

Acetonitrile, Orthophosphoric acid (OPA) and water (HPLC grade) were purchased from Merck (India) Ltd., Mumbai, India. API of Aceclofenac (purity-99.9%) and Misoprostol (purity-99.9%) as reference standards were procured from Glenmark Pharmaceutical private Ltd., Andheri (E), Mumbai, India.

**Equipment**

Chromatographic system of e-2695 with a quaternary pump, and a PDA detector of 2996 was used. The chromatographic data was analyzed with Empower software of version 2.0.

**Chromatographic Conditions**

By using the Chromatographic conditions separation was administered in isocratic mode at temperature employing a inertsil ODS (150mmx4.6mm, 3.5μ) column. The combination of 0.1 % orthophosphoric acid and acetonitrile 50:50 v/v with a flow 1ml/min was used as a mobile phase. The volume of injection was 10 μl and eluent was observed at 227 nm, so this was selected. The PDA spectrum of Aceclofenac and Misoprostol was shown in Figure 2.

**Figure 2: PDA spectrum of Aceclofenac and Misoprostol**

**Preparation of standard solution**

Weigh 50 mg of Aceclofenac and 50 mg of Misoprostol working standards and transferred into flask volume of 100 ml and diluted to volume with diluents. Further dilute 5 ml of the prepared solution to 50 ml with diluents.

**Preparation of sample solution**

Transfer 69.5 mg of Aceclofenac and Misoprostol sample into a flask of 100 ml and add 70 ml of diluents, sonicate to dissolve it and make up to the mark. Further dilute 5 ml of the prepared sample solution to 50 ml with diluents.

**OUTCOMES AND DEBATE**

**Method validation**

In this method validation parameter (system precision, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation and stability) studies were validated for the chosen drugs of Aceclofenac and Misoprostol. Standard and sample
| Table 1: Linearity data of Aceclofenac and Misoprostol |
|-----------------------------------------------|
| **Analyte** | **Range of Linearity** | **Calibration curve equation** | **Correlation coefficient** |
| Aceclofenac | 10-150 µg/ml | Y=68390.79x+14397.06 | 0.99998 |
| Misoprostol | 10-150 µg/ml | Y=70351.52x+4707.45 | 0.9999 |

| Table 2: Intraday precision data of Aceclofenac |
|-----------------------------------------------|
| **Analyte** | **Amount present** | **% assay (mean)** | **% RSD of assay** |
| Aceclofenac | 49.87±0.38 | 99.7 | 0.38 |
| Misoprostol | 49.93±0.21 | 99.5 | 0.46 |

| Table 3: Results of Accuracy study of Aceclofenac and Misoprostol |
|-----------------------------------------------|
| % of target concentration | **Aceclofenac** | **Misoprostol** |
| | **% Recovery** | **% RSD** | **% Recovery** | **% RSD** |
| 50 | 99.6 | 1.19 | 99.4 | 0.39 |
| 100 | 100.6 | 0.64 | 100.2 | 0.47 |
| 150 | 99.5 | 0.35 | 100.4 | 0.58 |

| Table 4: Robustness study of Aceclofenac and Misoprostol |
|-----------------------------------------------|
| **Drug name** | **Flow Plus** (1.2 ml/min) | **Flow Minus** (0.8 ml/min) | **Org Plus** (55:45) | **Org Minus** (45:55) |
| | % RSD | % RSD | % RSD | % RSD |
| Aceclofenac | 0.35 | 0.19 | 0.69 | 0.54 |
| Misoprostol | 0.27 | 0.62 | 0.42 | 1.36 |

| Table 5: Stability data of Aceclofenac and Misoprostol |
|-----------------------------------------------|
| **Time intervals** | **Aceclofenac (% Difference)** | **Misoprostol % Difference** |
| | | | |
| Initial | 0.00 | 0.00 |
| 6 Hrs | -0.9 | -0.7 |
| 12 Hrs | -1.1 | -0.9 |
| 18 Hrs | -1.7 | -1.5 |
| 24 Hrs | -2.8 | -2.2 |

| Table 6: Forced degradation data of Aceclofenac and Misoprostol |
|-----------------------------------------------|
| **Degradation** | **Aceclofenac (% degradation)** | **Misoprostol (% degradation)** |
| | | |
| Control | 0.1 | 0.2 |
| Acid degradation | 17.1 | 16.1 |
| Alkali degradation | 16.4 | 15.9 |
| Peroxide degradation | 14.6 | 13.5 |
| Reduction degradation | 12.7 | 12.7 |
| Thermal degradation | 13.2 | 10.8 |
| Hydrolysis degradation | 11.8 | 11.9 |
chromatograms of proposed technique were shown in Figures 3 and 4.

**System suitability**

The HPLC system was stabilized for 60 min to urge a stable bottom line. Six replicate injections of the quality solution containing 50 µg/ml of Aceclofenac and 50 µg/ml of Misoprostol were assessed to see the system suitability. The amount of the theoretical plate count of Aceclofenac and Misoprostol were 3265 and 6487, tailing factor was 1.04 and 1.25, resolution was 5.47 respectively. These parameters were found to be within the acceptable limit.

**Linearity**

To assess the tactic’s linearity, a standard solution containing 50 g/ml of Aceclofenac and 50 g/ml of Misoprostol was prepared (100 percent of the targeted level of the assay concentration). Sequential dilutions of the given solutions were performed at 10 percent, 25 percent, 50 percent, 100 percent, 125 percent, and 150 percent of the target concentrations. The peak areas are used to map calibration curves since they were pumped. These analytes had a correlation coefficient of 0.999. Table 1 summarized the conclusions and the Figures 5 and 6 shows the calibration plots of aceclofenac and misoprostol.

**Limit of detection and quantification**

Limit of detection and quantification is the minimum concentration level at which the analyte are often reliably detected, quantified by using the quality formulas ($3.3 \sigma/s$ and $10 \sigma/s$ for LOD and LOQ respectively). LOD value of Aceclofenac and Misoprostol were 0.06 µg/ml, 0.06 µg/ml and s/n values were 5, 5. LOQ value of Aceclofenac and Misoprostol were 0.208 µg/ml, 0.208 µg/ml and its s/n values were 26, 26.

**Precision**

The method precision of the process was investigated by examining six samples of an identical batch that were prepared separately. From these six separate samples, solution was injected and therefore the peak responses obtained went to calculate mean and percentage RSD values. This technique was observed to be precise and RSD was 2.0% and also the share assay values were on the brink of 100%. The results were represented in Table 2.

**Accuracy**

Accuracy decided by recovery studies which were administered in 3 individual concentration levels (50%, 100% and 150%). APIs with concentration 25, 50 and 75 g/ml were prepared. As per the test method the test solution was injected to 3 preparations at each spike level and therefore the assay was performed. The percentage recovery values were observed in between 99.5-100.6 of Aceclofenac and 99.4-100.4 of Misoprostol. The share recovery values were observed as 2%. The results were given in Table 3.

**Ruggedness**

The tactic’s sturdiness was investigated, and it was discovered that the chromatographic patterns did not change dramatically by using a different HPLC method, observer, or column. The fact that the RSD percentage was less than 2% demonstrates the robustness of the established process.
Robustness of the tactic was found to attract RSD should be but 2%. Slightly variations were wiped out the optimized method parameters like flow (±0.2 ml/min), organic content in mobile phase (±10%). The results were shown in Table 4.

Stability

By observing the stability techniques, stability of ordinary and sample solutions were studied from initial to 24 hrs stored at RT. They were injected at different time intervals and difference between initial to 24 hrs percentage of assay wasn’t quite 2%. There is no effect in storage conditions for Aceclofenac and Misoprostol drug. The results are shown below in Table 5.

Forced degradation

Forced degradation conditions containing acidic, basic, peroxide, hydrolysis, reduction and thermal stress were studied in 1 N concentration level. Forced degradation results were shown in Table 6.

CONCLUSION

This method describes the quantification of Aceclofenac and Misoprostol in bulk and pharmaceutical formulation according to ICH guidelines. The evolved technique was observed to be accurate, precise, linear and reliable. The benefit comes from the ease with which the sample was prepared, as well as the use of less expensive reagents. The proposed HPLC conditions ensure adequate resolution and, as a result, accurate compound quantification. The precision and reproducibility data are satisfactory, according to the testing results. The developed chromatographic technique was widely used in drug testing for routine study.

ACKNOWLEDGEMENT

The authors are thankful to Professor Dr. Kantipudi Rambabu for giving his valuable guidance and suggestions. I heartily thank full to Shree icon pharmaceutical laboratories, Vijayawada, India, for providing research facilities for this work.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

REFERENCES

Blum, J., Alfirevic, Z., Walraven, G., Weeks, A., Winikoff, B. 2007. Treatment of postpartum hemorrhage with misoprostol. International Journal of Gynecology & Obstetrics, 99(2):S202–S205.

Bouayad, A., Kajino, H., Waleh, N., Fouron, J.-C., Andelfinger, G., Varma, D., R., Skoll, A., Vazquez, A., Gobeil, F., Clyman, R. I., Chemtob, S. 2001. Characterization of PGE2 receptors in fetal and newborn lamb ductus arteriosus. American Journal of Physiology-Heart and Circulatory Physiology, 280(5):H2342–H2349.

Bryant, A. G., Regan, E., Stuart, G. 2014. An Overview of Medical Abortion for Clinical Practice. Obstetrical & Gynecological Survey, 69(1):39–45.

Cherem, J. H., Malagon, J., Nellen, H. 2005. Cimetidine and Acute Intermittent Porphyria. Annals of Internal Medicine, 143(9):694–694.

Glyn-Jones, S., Palmer, A. J. R., Agricola, R., Price, A. J., Vincent, T. L., Weinans, H., Carr, A. J. 2015. Osteoarthritis. The Lancet, 386(9991):376–387.

Isacs, H. 2008. Fetal Hydrops Associated with Tumors. American Journal of Perinatology, 25(1):43–68.

Kramer, M. S., Kakuma, R. 2012. Optimal duration of exclusive breastfeeding. Cochrane Database of Systematic Reviews, 8(8).

Kulier, R., Kapp, N., Gülmezoglu, A. M., Hofmeyr, G. J., Cheng, L., Campana, A. 2004. Medical methods for first trimester abortion. Cochrane database of systematic reviews, (1).

Machado, G. C., Maher, C. G., Ferreira, P. H., Day, R. O., Pinheiro, M. B., Ferreira, M. L. 2017. Nonsteroidal anti-inflammatory drugs for spinal pain: a systematic review and meta-analysis. Annals of the Rheumatic Diseases, 76(7):1269–1278.

Mantovani, A., Allavena, P., Sica, A., Balkwill, F. 2008. Cancer-related inflammation. Nature, 454(7203):436–444.

Marret, H., Simon, E., Beucher, G., Dreyfus, M., Gaudineau, A., Vayssière, C., Lesavre, M., Pluchon, M., Winer, N., Fernandez, H., Aubert, J., Bejan-Angoulvant, T., Jonville-Bera, A. P., Clouqueur, E., Houfflin-Debarge, V., Garrigue, A., Pierre, F. 2015. Overview and expert assessment of off-label use of misoprostol in obstetrics and gynaecology: review and report by the Collège national des gynécologues obstétriciens français. European Journal of Obstetrics & Gynecology and Reproductive Biology, 187:80–84.

Murphy, D. J. 2006. Uterine rupture. Current Opinion in Obstetrics & Gynecology, 18(2):135–140.
Raymond, E. G., Harrison, M. S., Weaver, M. A. 2019. Efficacy of Misoprostol Alone for First-Trimester Medical Abortion. Obstetrics & Gynecology, 133(1):137–147.

Ricciotti, E., FitzGerald, G. A. 2011. Prostaglandins and Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology, 31(5):986–1000.

Smith, J. A. 2015. Update on Ankylosing Spondylitis: Current Concepts in Pathogenesis. Current Allergy and Asthma Reports, 15(1):489–489.

Smolen, J. S., Aletaha, D., McInnes, I. B. 2016. Rheumatoid arthritis. The Lancet, 388(10055):2023–2038.

Stein, P. E., Badminton, M. N., Rees, D. C. 2017. Update review of the acute porphyrias. British Journal of Haematology, 176(4):527–538.

Winkler, N. S., Fautsch, M. P. 2014. Effects of Prostaglandin Analogues on Aqueous Humor Outflow Pathways. Journal of Ocular Pharmacology and Therapeutics, 30(2-3):102–109.