Macedonian pharmaceutical bulletin, 69 (Suppl 1) 223 - 224 (2023)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2023.69.03.109
Short communication

Application of chromatography modeling technologies in development of method for related and degradation products in drug product containing two active ingredients

Maja Hadzieva Gigovska*, Oja Ali Memed, Ivana Klinkarova, Packa Antovska, Jelena Lazova

Research and development, Alkaloid AD Skopje, Aleksandar Makedonski 12, 1000, Skopje, Republic of North Macedonia

Introduction

Chromatography modeling is a new, but no widely implemented knowledge-oriented trend in modern pharmaceutical analysis. The traditional way of chromatographic method development is based on the empirical trial-and-error approach, which often results in a lack of adequate process understanding and unforeseen difficulties during method validation (Mattrey et al., 2017). Due to the variety of parameters such as stationary phase, pH, composition of mobile phase and column temperature, finding the optimum conditions for the analysis normally requires substantial efforts both in experimental work and result evaluation, which is often tedious, time-consuming work than involves considerable cost. Therefore, a need for more systematic approach is recognized.

In this paper, a novel high-pressure liquid chromatographic (HPLC) impurity profiling method is reported for drug product containing two active pharmaceutical ingredients (API), and the benefits of chromatography modeling in method development is discussed. The proposed method is intended for simultaneous determination of API component 1 and its impurities (A, B, C, D, E and F) and API component 2 and its Ph.Eur. impurities (A, B and G).

According to the literature review there are few methods for the estimation of API-1 (Housheh et al,2017) and API-2 (Shah et al.2022) in drug product containing two active pharmaceutical ingredients; but most of them failed to demonstrate the separation of API-1 and API-2, and all other nine impurities.

To the best of our knowledge, application of chromatography modeling technologies in development HPLC separation of all the related impurities for both APIs was not reported up to now. Hence, it is imperative to develop a HPLC method for the simultaneous estimation of API-1, API-2, and their impurities in drug product containing two active pharmaceutical ingredients using the advantages of chromatography modeling.

Materials and methods

Acetonitrile (gradient grade), formic acid, ammonium formate and triethylamine were purchased from Merck (Darmstadt, Germany). All reagents used were of analytical grade. API component 1 and its impurities (A, B, C, D, E and F) were purchased from Veeprho Pharmaceuticals, Czech Republic. API component 2 and its Ph.Eur. impurities (A, B and G) were purchased from European Directorate for the Quality of Medicines and Health Care (EDQM).

Experiments were carried on Agilent Infinity II HPLC System 1260 equipped with a binary gradient pump. Prior to the retention modeling, preliminary screening experiments were performed for the selection of stationary phase and organic modifiers. Based on obtained results, gradient steepness (tG), temperature (T) and mobile phase pH with 12 input runs were designed to build a model for achieving the optimized separation. Modeling was based on the measurement of retention times and peak areas using DryLab v.4.0 (Molnár-Institute, Berlin, Germany). The criteria for the proposed model were: impurities have to be separated from (a) each other, (b) the APIs, and (c) other

*mhadzieva@alkaloid.com.mk

possible disturbing compounds. For the baseline separation of the critical peak pairs, the value of critical resolution (Rs,crit) should be higher than 1.5.

Simultaneous determination of related substances was performed on Inifinity Poroshell C1(Agilent Technologies. Inc, USA) column (100 × 2.1 mm; 1.9 μm particle size) at 2 different temperatures (35°C and 55°C) employing a gradient elution (for 20 and 60 minutes). The mobile phase A consisting of 20 mM ammonium formate buffer (pH 2.8, 3.8, and 4.8) with added 1 mL triethylamine as modifier and the mobile phase B consisting of acetonitrile was used

For the model runs, the mobile phase flow rate was set to 0.2 mL min^{-1} and gradients were run from 5 to 95 % acetonitrile. The injection volume was set to 1 μL UV detection was performed at 230 nm.

The experiments were performed on a sample with concentration of 1 mg/mL of both APIs, spiked with their impurities at 0.1 % level. Sample solvent was a mixture of buffer solution pH 4.8 and acetonitrile in ration = 90:10 (v/v).

Results and discussion

Chromatographic behavior of chosen model compounds and their related substances were examined using DryLab v.4.0 based on prior knowledge. They exhibit a wide variety of different chromatographic properties; therefore, they were selected for the experiments as model compounds.

API-1 and its impurities were found to be relatively lipophilic. API-2 Imp. A was found to be highly lipophilic, therefore high organic content, i.e., over 95% was required the end of the gradient to elute this substance, hence the starting mobile phase composition was set to 5%. Furthermore, there was a structural similarity between API-2, API-2 Imp.D, API-2 Imp.E and API-2 Imp. F as all of them contained a basic primary amino group (pKa > 10), meaning that all these substances were assumed to be ionized under common reversed phase conditions.

There are several basic and acidic groups in the structures of all evaluated impurities, which suggest to investigate the influence of the pH, the gradient steepness and the temperature. Experimental design for simultaneous optimization of gradient time (tG), pH, and ternary composition (tC) required 12 experiments. With data generated from only 12 input experiments, DryLab create a multi-dimensional resolution map, showing the critical resolution of the peaks to be separated against the three factors wherefrom the set of conditions for above mention desired criteria was chosen.

Furthermore, the software predicts how changes in many additional method parameters affect separation.

Analyzing the obtained results, it could be concluded that the alteration of pH between 2.8 and 4.8 and the effect of changes in % B had small effects, the retention times not changed remarkably, as long the change in temperature had significant effects on the retention and critical resolution of the components.

As a next step, the accuracy of the predicted results was evaluated. Experimental verifications of predicted chromatograms were performed. The results showed a satisfactory agreement between the predicted and experimental values, with average errors of 1.74% and 12.19% for retention time and resolution, respectively.

The applicability of this method was evaluated by analyzing samples of commercially available tablets. The specificity was established by demonstrating that there is no interference between peaks of interest and peaks from diluent and placebo.

Application of chromatography modeling, allowed finding suitable separation (Rs,crit > 1.5) for 13 components, by proper adjustments of gradient, temperature and pH, with a minimum number of experiments. Chromatographic modelling software not only speed up the analytical method development process, but also improve the reliability of the developed method.

Conclusion

This paper highlights significant utility of chromatography modeling in optimization of method development especially for related and degradation products of drug product containing two active ingredients. The proposed methodology represents an efficient and easily accomplishable approach for resolving the problem of searching the optimum chromatographic conditions in minimum time.

This study showed that chromatographic modeling provides useful information of separation and elution time, making this combined technique a powerful analytical tool.

References

Mattrey, F.T., Makarov, A.A., Regalado, E.L., Bernardoni, F., Figus, M., Hicks, M.B., Zheng, J., Wang, L., Schafer, W., Antonucci, V., Hamilton, S.E., Zawatzky, K., Welch, C.J.,2017. Current challenges and future prospects in chromatographic method development for pharmaceutical research. Trends in Analytical Chemistry. 95, 36–46. doi: 10.1016/j.trac.2017.07.021

Shah, J.P., Akabari, A.H., Patel, S., Surati, J., 2022. Amlodipine: A Review of Characteristics, Properties, Analytical Methods and Impact in the Green Chemistry. International Journal of Pharmaceutical Science Invention. 11, 30–38. doi: 10.35629/6718-11033038

Housheh, S., Jdeed, L., 2017. Analytical methods of perindopril, review. World Journal of Pharmaceutical Research. 6, 1563-1575. doi: 10.20959/wjpr20174-8268