

Optimization of liquid chromatographic separation of pharmaceuticals within green analytical chemistry framework

Aysegul Dogan^{a,b}, Marek Tobiszewski^{a,*}

^a Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology (GUT), 11/12 G. Narutowicza St., Gdańsk 80-233, Poland

^b Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, Sıhhiye, Ankara 06100, Turkey



ARTICLE INFO

Keywords:

Design of experiment
Desirability function
Multi-objective optimization
Non-Steroid Anti-Inflammatory Drugs
Greenness Assessment

ABSTRACT

The contribution is aimed at the development of methodology that allows to consider green analytical chemistry criteria during optimization of liquid chromatographic separation with design of experiment. The objectives of the optimization are maximization of peak areas of five non-steroid anti-inflammatory drugs, maximization of resolution between peaks, with simultaneous shortening of chromatographic separation time and minimization of mobile phase environmental impact. This is obtained with design of experiment to consider many experimental conditions and Derringer's desirability function to combine many optimization objectives. The possibilities of introduction different green analytical chemistry metrics are discussed and the methodology of mobile phase greenness assessment is proposed. The optimal response for all objectives is obtained for 0.96 mL min⁻¹ of mobile phase flow rate, 61% of MeOH content, temperature of 25 °C and pH equal to 4.5. The separation takes less than 9 min.

1. Introduction

Green Analytical Chemistry was (GAC) emerged from green chemistry in 2000 [1]. This approach to green chemistry aims to make laboratory applications of analytical chemists more environmentally friendly [2–4]. In addition to the developments in method and device technologies, the efforts to reduce the negative effects of chemicals on the environment and the applications of sustainable development to the analytical chemistry laboratories are becoming increasingly important. In this context, GAC is considered as one of stimulants of analytical chemistry development. One of the important approaches in this discipline is the increase in environmentally friendly studies in parallel with the increase in the quality of analytical results. The main strategies of greening analytical chromatography are shortening of analysis time (by increasing pressure or temperature), reduction of columns dimensions, finding of greener solvents as mobile phase constituents and recycling of mobile phase [5–8].

The GAC principles were derived from Anastas and Werner's green chemistry principles by Galuszka et al. [9]. Among these GAC principle's minimizing sample size and samples number can be found for the optimization step of the experiments. Chemometric and statistical techniques can be applied for optimization besides conventional techniques. Changing one variable at a time for a multi-analyte matrix can lead the analyst to make more experiments than it is needed in case of

chemometric optimization. From GAC point of view, in the number of optimization experiments gets higher, the amount of chemicals used for the analysis will increase.

In order to determine the optimum conditions, the classical method, in which the single variable is changed in each step, may require a lot of experimental action, especially if many variables influence the experimental conditions, and the experiments become exhausting, tedious and time consuming. The main purpose of design of experiments (DOE) application is to express the correlation between the input and output variables. DOE can be used for comparison, variable screening, transfer function identification, system optimization, and robust design [10].

DOE is not a statistical technique only it is also leading the analyst to make better and efficient optimization process. DoE becomes an integral part of optimization, especially when multiple variables are considered at the same time. The effect of each variable is indispensable to the experimental conditions where the experimental design also depends on other variables. With experimental design optimization, both the optimum values of the factors affecting the experimental result are found and the mathematical model showing the effect of each factor on the result is created. Optimization is the process of determining the optimum values of the important factors found by screening.

A problem that arises in the chromatographic optimization steps is the choice of conditions that give the desired combination of chromatographic parameters. This is a problem involving simultaneous

* Corresponding author.

E-mail addresses: martobis@pg.edu.pl, marektobiszewski@wp.pl (M. Tobiszewski).

<https://doi.org/10.1016/j.microc.2019.104323>

Received 1 September 2019; Received in revised form 7 October 2019; Accepted 8 October 2019

Available online 14 October 2019

0026-265X/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

optimization of multiple response variables. These variables can be contradictory to each other. The principle of the desirability function involves the aggregation of all responses into a single function defined as the “desirability function”. Among the advantages of this method are the ability to analyze responses of different units and scales together, ease of conversion of the responses into a single function and the possibility to use qualitative and quantitative responses. The desirability function developed by Derringer and Suich converts each response variable into a numerical value [11]. The desirability value is in the range of $0 \leq d_i \leq 1$. When the values of initial responses approach the optimal range, the corresponding desirability value d_i increases, with optimal values transformed to $d_i = 1$. Then the geometric mean, called global desirability D is calculated from all individual desirability values. Global desirability D takes a values in the range 0–1, and when the characteristics are more desirable, the value of D increases. When one of the responses of variables is not acceptable then its desirability equals 0 and global desirability also equals 0. The most important advantage of the desirability function approach over the above-mentioned approaches is that it allows simultaneous maximization and/or minimization of a multiple responses [12,13]. In addition, this approach allows one to include expert’s/analyst’s knowledge of the significance of each response in a subjective way.

There are several analytical methodologies, which applied the optimization process using Derringer’s desirability function methodology in case of liquid chromatographic pharmaceutical analysis [14–29]. In this study Non-Steroid Anti-Inflammatory drugs (NSAID), which are highly prescribed and consumed as pain relievers worldwide subjected to chromatographic analysis. Many members of this group are also marketed as “Over The Counter”, which also contributes to its consumption due to the convenience of reaching from the market shelf directly. NSAIDs are generally used for their analgesic, antipyretic and anti-inflammatory effects. NSAIDs divide into three different groups as acidic, non-acidic and coxib. The analytes chosen were ibuprofen, ketoprofen, fenoprofen, naproxen and diclofenac, which are the acidic members of this group of drugs. Their ease of purchasing and the popularity among most of the population resulted in finding in many samples in quantitative concentrations. Their simultaneous analysis is needed in case environmental and biological analysis. There are plenty of methodologies developed for the analysis of these drugs in the literature, which all need to be optimized in the first step because of their similar chromatographic activities. Optimum conditions to separate five peaks with acceptable chromatographic parameters were determined using the Derringer multi-criteria response technique.

The aim of the study is to develop the methodology of multi-objective optimization of liquid chromatographic separation that allows to include GAC principles. To obtain this compromise DoE, Derringer’s desirability functions and GAC metrics will be applied.

2. Materials and methods

2.1. Chemicals

Methanol, phosphoric acid and ammonia solutions (25%) were of HPLC grade and purchased from Sigma Aldrich (Germany). Ultrapure water used was obtained with a Milli-Q Gradient A10 System (Millipore, Bedford, MA, USA). Buffer solutions of pH 4.01, 7.01 and 10.01 were used for pH meter calibration. Ibuprofen, ketoprofen, fenoprofen, naproxen and diclofenac were purchased from Sigma Aldrich (Germany). Analytes stock solutions (1000 $\mu\text{g}/\text{mL}$) were prepared in MeOH and working solutions used in 29 analyses were diluted from stock solutions using MeOH. The final concentration of these analytes used in the analysis was 10 $\mu\text{g mL}^{-1}$.

2.2. Instrumentation and chromatographic conditions

Experiments were carried out using an Agilent 1100 HPLC system

Table 1

The variables and their levels used in the experimental plan.

Level	A: Flow Rate [mL min^{-1}]	B: Organic solvent (Methanol) [%]	C: Temperature [°C]	D: pH
–1	0.80	55	20	2.5
0	1.00	65	25	4.5
1	1.20	75	30	6.5

consisting of degasser (G1379B), binary pump (G1312A), autosampler (G1313A), column oven (G1316A), diode array detector (G1315A), and Agilent Chemstation software as data analyser. All the analytes were monitored at 230 nm wavelengths. Mchenary-Neigel Nucleosil C18 (150 \times 4.6 mm, 5 μm) column was used to perform the analysis. The mobile phase consisted of methanol:water in different ratios of organic solvent as stated in Table 1.

2.3. Design of experiment

DoE is used to optimize the flow rate of mobile phase, the content of MeOH in mobile phase and its temperature and pH for separation of NSAID. It is designed for these four factors as it is shown in the Table 1. The variables and their boundary values applied in DoE were based on own experience and typical literature values for similar LC separations.

Table 2 presents the structure of central design plan. All the calculations to obtain the optimal values with central composite design plan are performed with Statistica software. The order of the experimental repetitions was set to be random, to minimize any influence of bias errors.

2.4. Derringer’s desirability function

Desirability function was applied to perform optimization with multiple objectives. These multiple objectives are large peak areas (5 objectives), good resolution between peaks (4 objectives), retention

Table 2

Experimental design plan.

Experiment	A: Flow Rate [mL min^{-1}]	B: Organic solvent (Methanol) [%]	C: Temperature [°C]	D: pH
29 (C)	0	0	0	0
7	–1	1	1	–1
20	0	1	0	0
5	–1	1	–1	–1
19	0	–1	0	0
9	1	–1	–1	–1
22	0	0	1	0
15	1	1	1	–1
6	–1	1	–1	1
26 (C)	0	0	0	0
21	0	0	–1	0
28 (C)	0	0	0	0
2	–1	–1	–1	1
18	1	0	0	0
14	1	1	–1	1
11	1	–1	1	–1
17	–1	0	0	0
4	–1	–1	1	1
8	–1	1	1	1
24	0	0	0	1
3	–1	–1	1	–1
16	1	1	1	1
12	1	–1	1	1
10	1	–1	–1	1
13	1	1	–1	–1
27 (C)	0	0	0	0
1	–1	–1	–1	–1
23	0	0	0	–1
25 (C)	0	0	0	0

time of last eluting compound and low environmental impact of mobile phase for all analytes at the same time. This gives total number of 11 objectives that are included in optimization process. For each of criteria completely desirable response ($d = 1$) and completely undesirable response ($d = 0$), and desirability function equation are assumed. In all cases linear functions between desirable and undesirable response are assumed. The assumption is the desirable response ($d = 1$) the most preferential value from all chromatographic runs. The undesirable response ($d = 0$) is the least preferential value of the response. The next step in application of Derringer's desirability function is combination of desirabilities for respective criteria into global desirability (D) as it is shown with Eq. (1). If any of criteria has undesirable response ($d = 0$), then global desirability is also undesirable ($D = 0$). Derringer's desirability function allows to assign different relative importance to criteria ($r_1, r_2 \dots r_n$) but here we apply equal importance for all criteria.

$$D = (d_1^{r_1} \times d_2^{r_2} \times \dots \times d_n^{r_n})^{\frac{1}{\sum r_i}} \quad (1)$$

Global desirability function that is calculated for all chromatographic runs is the input data to draw response surface for optimization of four variables relevant to liquid chromatographic process. Desirability functions and global desirabilities were calculated in Excel program.

3. Results and discussion

3.1. Mobile phase greenness assessment

Implementation of proper greenness metric system in DoE is crucial for this study. NEMI labelling system [11,30], probably the most widely accepted metric system, is not suitable to be combined with DoE. It does not consider the amount of solvent used in the procedure, so the differences in greenness for respective chromatographic runs would not be noticed. Another greenness metric system that is in common use is analytical Eco-scale [31]. This tool includes the amount of solvent in the range below 10 mL, between 10 and 100 mL and above 100 mL. Therefore, it is not applicable as the amount of solvent used in all chromatographic runs falls into one range. It is similar to recently developed tool – Green Analytical Procedure Index (GAPI) [32], where solvent consumptions in all experimental conditions are in single range.

It is clear that for the purpose of this study it is needed to apply a tool that considers the volume of solvent in continuous way, to see any changes in the greenness resulting from varying experimental conditions. The metric system that has been selected is based on the type of solvent and the volume that is applied. The simple algorithm allows to calculate total analytical hazard value that is a combination of few hazard related and exposure related parameters [33]. Finally, each solvent is assessed with numerical value ranging from 2.6 for acetone to 122 for benzene and 125.1 for trichloroethene. With this tool, first it was established to apply methanol (score 15.7) instead of acetonitrile (score 26.8) as mobile phase constituent. This greenness metric system supports the statements of the others that methanol is greener solvent and mobile phase constituent over acetonitrile [34,35]. The same sources state that acetone is in similar greenness range as MeOH. For the assessment of analytical methodologies the calculated score is multiplied by the volume of solvent applied.

To assess respective chromatographic runs total analytical hazard values (taHV) multiplied by volume of solvent were the input data to calculate desirability functions further applied in DoE. This was calculated according to formulae:

$$EI = \text{MeOH taHV} \times \text{MeOH} \% \times \text{FR} \times (\text{RTn} + 0.5) \quad (2)$$

Where EI is environmental impact of chromatographic run, MeOH taHV is 15.7 calculated according to [29]. In this case it is constant and therefore could be omitted but it is important for generalized form of EI equation. MeOH% is methanol content in mobile phase, FR is its flow

Table 3
Desirability functions calculated for all experimental conditions.

Experiment	A: flow rate [ml min ⁻¹]	B: Methanol [%]	C: T [°C]	D: pH	D
29 (C)	1	65	25	4.5	0.457
7	0.8	75	30	2.5	0.347
20	1	75	25	4.5	0.389
5	0.8	75	20	2.5	0.410
19	1	55	25	4.5	0.610
9	1.2	55	20	2.5	0.436
22	1	65	30	4.5	0.522
15	1.2	75	30	2.5	0.103
6	0.8	75	20	6.5	0.574
26 (C)	1	65	25	4.5	0.594
21	1	65	20	4.5	0.547
28 (C)	1	65	25	4.5	0.534
2	0.8	55	20	6.5	0
18	1.2	65	25	4.5	0
14	1.2	75	20	6.5	0
11	1.2	55	30	2.5	0
17	0.8	65	25	4.5	0.499
4	0.8	55	30	6.5	0.419
8	0.8	75	30	6.5	0.393
24	1	65	25	6.5	0
3	0.8	55	30	2.5	0
16	1.2	75	30	6.5	0
12	1.2	55	30	6.5	0
10	1.2	55	20	6.5	0
13	1.2	75	20	2.5	0
27 (C)	1	65	25	4.5	0.237
1	0.8	55	20	2.5	0
23	1	65	25	2.5	0.275
25 (C)	1	65	25	4.5	0.347

Table 4
Basic metrological parameters of chromatographic determination of the NSAID.

	LOD [mg L ⁻¹]	LOQ [mg L ⁻¹]	CV _{RT} [%] (n = 7)	CV [%] (n = 3)
Ketoprofen	2.1	7.1	0.38	8.8
Naproxen	0.15	0.50	0.19	4.6
Fenoprofen	1.1	3.6	0.15	12
Diclofenac	0.9	3.0	0.18	7.3
Ibuprofen	1.2	4.0	0.10	11

rate and RTn is the retention time of last eluting peak with 0.5 min of time excess as the analysis cannot be stopped at the time of elution of last peak. The volume of water was ignored as it is assumed it does not contribute to environmental impact. The equation for more than one problematic mobile phase constituents has the following form:

$$EI = (A \text{ taHV} \times A \% + B \text{ taHV} \times B \% + \dots + X \text{ taHV} \times X \%) \times \text{FR} \times (\text{RTn} + 0.5) \quad (3)$$

Where A, B and X are different compounds being mobile phase constituents. The equation is valid for isocratic elution but the approach is fully applicable for gradient elution. In such case the volumes of all problematic mobile phase constituents should be calculated. EI values are incorporated to calculate Derringer's desirability functions as one of the optimisation goals.

3.2. Combination of greenness assessment with DoE

Based on experimental plan different peak areas, resolutions, separation times and environmental impact of mobile phase are obtained for all 29 chromatographic runs. They are recalculated to global desirability as presented in Table 3.

Undesirable values of global desirability is mainly achieved for chromatographic runs with flow rate of 1.2 mL min⁻¹ and low MeOH contents of 55%. In such conditions no proper resolution is achieved.

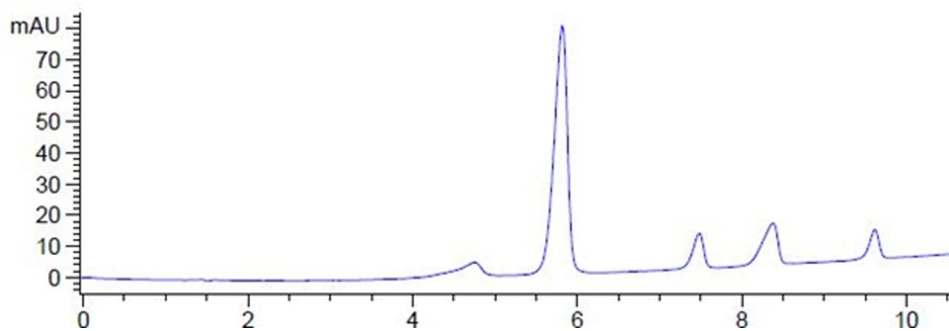


Fig. 1. Chromatogram obtained in the optimized conditions. Peaks of ketoprofen, naproxen, fenoprofen, diclofenac, ibuprofen, respectively.

The values of pKa are ketoprofen = 4.45, naproxen = 4.15, fenoprofen = 4.5, diclofenac 4.15 and ibuprofen = 5.3. The obtained optimal value for pH is 4.5, while it is in contradiction with pKa value of ibuprofen. This could be the explanation for quite poor CV value and high values of LOD and LOQ (as presented in Table 4).

3.3. Optimal conditions

Under optimal conditions the levels of variables are 0.96 mL min⁻¹ of mobile phase flow rate, 61% of MeOH content, temperature of 25 °C and pH equal to 4.5. The chromatogram obtained in these optimized conditions is presented in the Fig. 1. All the peaks are well resolved, they are characterized by larger peak areas and the consumption of MeOH is low while chromatographic separation is short.

The values of precision (expressed as CV), CV of peak retention time, LODs and LOQs are presented in Table 4. The values of LOD and LOQ are in at very good levels, while the values of precision are at acceptable (CV = 4.6 – 12%) levels.

To our best knowledge the direct incorporation of green analytical chemistry principles has not been done in case of liquid chromatography separation process. The literature studies show that environmental impact of solvents applied in dispersive liquid-liquid micro-extraction technique has been assessed and optimized. In this case multiple optimization objectives were high responses of nine chlorophenols simultaneously with low environmental impact of the applied amounts solvents. Volumes of water sample, dispersive solvent and extraction solvents were optimized with DoE and the objectives were combined with Derringer's desirability function [36].

Another practical approaches towards making analytical chromatography greener are based on scoring systems [37,38]. In these approaches mobile phase constituents are assessed by multiplying the mass of solvent in single chromatographic run by scores related to environmental, health and safety hazards. These scoring systems are equally applicable to the approach presented here.

4. Conclusion

DoE was applied to optimize the process of liquid chromatographic separation of pharmaceuticals. The presented approach enables to perform less experiments for optimization step than it is in the conventional optimization process of changing one variable at a time. The calculation of the desirability function allows to introduce multi-objective approach to the optimization process as many parameters are combined into single score. In this case the resolution between analytes peaks, chromatographic responses, chromatographic separation time were expressed as global desirability. The presented approach allows to integrate the green analytical chemistry aspect as one of optimization criteria as one of optimization objectives is minimization of toxic solvents utilization. The presented methodology is simple, as it applies very commonly used tools in slightly modified form and requires significantly low number of chromatographic runs.

Declaration of Competing Interest

Authors declare no conflict of interest.

Acknowledgements

The Scientific and Technological Research Council of Turkey (TUBITAK) is acknowledged for granting of Asst. Prof. Aysegül DOĞAN in the framework of TUBITAK-BİDEB 2219-International Postdoctoral Research Scholarship Program. We would like to thank Dr. Weronika Hewelt-Belka for her kind technical assistance.

References

- [1] J. Namieśnik, Trends in environmental analytics and monitoring, Crit. Rev. Anal. Chem. 30 (2000) 221–269 <https://doi.org/10.1080/10408340091164243>.
- [2] P.T. Anastas, Green chemistry and the role of analytical methodology development, Crit. Rev. Anal. Chem. 29 (1999) 167–175 <https://doi.org/10.1080/10408349891199356>.
- [3] M. Koel, M. Kaljurand, Application of the principles of green chemistry in analytical chemistry, Pure Appl. Chem. 78 (2006) 1993–2002 <https://doi.org/10.1351/pac200678111993>.
- [4] M. Tobiszewski, A. Mechlińska, J. Namieśnik, Green analytical chemistry—theory and practice, Chem. Soc. Rev. 39 (2010) 2869–2878 <https://doi.org/10.1039/B926439F>.
- [5] C.J. Welch, N. Wu, M. Biba, R. Hartman, T. Brkovic, X. Gong, ... L. Zhou, Greening analytical chromatography, TrAC Trends Anal. Chem. 29 (7) (2010) 667–680 <https://doi.org/10.1016/j.trac.2010.03.008>.
- [6] J. Plotka, M. Tobiszewski, A.M. Sulej, M. Kupka, T. Gorecki, J. Namieśnik, Green chromatography, J. Chromatogr. A 1307 (2013) 1–20 <https://doi.org/10.1016/j.chroma.2013.07.099>.
- [7] H. Shaaban, New insights into liquid chromatography for more eco-friendly analysis of pharmaceuticals, Anal. Bioanal. Chem. 408 (25) (2016) 6929–6944 <https://doi.org/10.1007/s00216-016-9726-2>.
- [8] H.M. Mohamed, Green, environment-friendly, analytical tools give insights in pharmaceuticals and cosmetics analysis, Trends Anal. Chem. 66 (2015) 176–192 <https://doi.org/10.1016/j.trac.2014.11.010>.
- [9] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the significance mnemonic of green analytical practices, Trends Anal. Chem. 50 (2013) 78–84 <https://doi.org/10.1016/j.trac.2013.04.010>.
- [10] D.B. Hibbert, Experimental design in chromatography: a tutorial review, J. Chromatogr. B 910 (2012) 2–13 <https://doi.org/10.1016/j.jchromb.2012.01.020>.
- [11] G. Derringer, R. Suich, Simultaneous optimization of several response variables, J. Qual. Tech. 12 (1980) 214–219 <https://doi.org/10.1080/00224065.1980.11980968>.
- [12] M.A. Bezerra, S.L.C. Ferreira, C.G. Novaes, A.M.P. dos Santos, G.S. Valasques, U.M. da Mata Cerqueira, J.P. dos Santos Alves, Simultaneous optimization of multiple responses and its application in analytical chemistry—a review, Talanta 194 (2019) 941–959 <https://doi.org/10.1016/j.talanta.2018.10.088>.
- [13] L.V. Candiotti, M.M. De Zan, M.S. Camara, H.C. Goicoechea, Experimental design and multiple response optimization. Using the desirability function in analytical methods development, Talanta 124 (2014) 123–138 <https://doi.org/10.1016/j.talanta.2014.01.034>.
- [14] N.H. Abou-Taleb, D.R. El-Wasseef, D.T. El-Sherbiny, S.M. El-Ashry, Multiobjective optimization strategy based on desirability functions used for the microemulsion liquid chromatographic separation and quantification of norfloxacin and tinidazole in plasma and formulations, J. Sep. Sci. 38 (2015) 901–908 <https://doi.org/10.1002/jssc.201401203>.
- [15] K. Abu-Izza, L. Garcia-Contreras, D.R. Lu, Preparation and evaluation of zidovudine-loaded sustained-release microspheres. 2. Optimization of multiple response variables, J. Pharm. Sci. 85 (1996) 572–576 <https://doi.org/10.1021/js960021k>.

- [16] P. Barmपालेखिस, F.I. Kanaze, E. Georganakis, Developing and optimizing a validated isocratic reversed-phase high-performance liquid chromatography separation of nimodipine and impurities in tablets using experimental design methodology, *J. Pharm. Biomed. Anal.* 49 (2009) 1192–1202 <https://doi.org/10.1016/j.jpba.2009.03.003>.
- [17] M. Cruz-Monteagudo, F. Borges, M.N.D. Cordeiro, Desirability-based multiobjective optimization for global QSAR studies: application to the design of novel NSAIDs with improved analgesic, antiinflammatory, and ulcerogenic profiles, *J. Comput. Chem.* 29 (2008) 2445–2459 <https://doi.org/10.1002/jcc.20994>.
- [18] P. Cutroneo, M. Beljean, R.P.T. Luu, A.-M. Siouffi, Optimization of the separation of some psychotropic drugs and their respective metabolites by liquid chromatography, *J. Pharm. Biomed. Anal.* 41 (2006) 333–340 <https://doi.org/10.1016/j.jpba.2005.10.050>.
- [19] R. Djang'eing'a Marini, P. Chiap, B. Boulanger, W. Dewe, P. Hubert, J. Crommen, LC method for the simultaneous determination of R-timolol and other closely related impurities in S-timolol maleate: optimization by use of an experimental design, *J. Sep. Sci.* 26 (2003) 809–817 <https://doi.org/10.1002/jssc.200301367>.
- [20] P. Giriraj, T. Sivakkumar, Development and validation of a rapid chemometrics assisted RP-HPLC with PDA detection method for the simultaneous estimation of pyridoxine HCl and doxylamine succinate in bulk and pharmaceutical dosage form, *Chromatogr. Res. Int.* 2014 (2014), <http://dx.doi.org/10.1155/2014/827895>.
- [21] P. Giriraj, T. Sivakkumar, A rapid-chemometrics assisted RP-HPLC method with PDA detection for the simultaneous estimation of ofloxacin and nimorazole in pharmaceutical formulation, *J. Liq. Chromatogr. Related Technol.* 38 (2015) 904–910 <https://doi.org/10.1080/10826076.2014.991870>.
- [22] N. Hatambeygi, G. Abedi, M. Talebi, Method development and validation for optimised separation of salicylic, acetyl salicylic and ascorbic acid in pharmaceutical formulations by hydrophilic interaction chromatography and response surface methodology, *J. Chromatogr. A* 1218 (2011) 5995–6003 <https://doi.org/10.1016/j.chroma.2011.06.009>.
- [23] V.S. Janardhanan, R. Manavalan, K. Valliappan, Chemometric technique for the optimization of chromatographic system: simultaneous HPLC determination of rosuvastatin, telmisartan, ezetimibe and atorvastatin used in combined cardiovascular therapy, *Arab. J. Chem.* 9 (2016) S1378–S1387 <https://doi.org/10.1016/j.arabj.2012.03.001>.
- [24] B. Otašević, S. Milovanović, M. Zečević, J. Golubović, A. Protić, UPLC method for determination of moxonidine and its degradation products in active pharmaceutical ingredient and pharmaceutical dosage form, *Chromatographia* 77 (2014) 109–118 <https://doi.org/10.1007/s10337-013-2580-x>.
- [25] T. Sivakumar, R. Manavalan, C. Muralidharan, K. Valliappan, Multi-criteria decision making approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole, *J. Pharm. Biomed. Anal.* 43 (2007) 1842–1848 <https://doi.org/10.1016/j.jpba.2006.12.007>.
- [26] T. Sivakumar, R. Manavalan, C. Muralidharan, K. Valliappan, An improved HPLC method with the aid of a chemometric protocol: simultaneous analysis of amlodipine and atorvastatin in pharmaceutical formulations, *J. Sep. Sci.* 30 (2007) 3143–3153 <https://doi.org/10.1002/jssc.200700148>.
- [27] R.S. Sundar, K. Valliappan, An improved RP-HPLC method for the simultaneous estimation of aspirin, atorvastatin, and clopidogrel in pharmaceutical formulation using experimental design methodology, *Int. J. Pharm. Pharm. Sci.* 6 (2014) 279–283.
- [28] R. Suresh, R. Manavalan, K. Valliappan, Developing and optimizing a validated RP-HPLC method for the analysis of amlodipine and ezetimibe with atorvastatin in pharmaceutical dosage forms applying response surface methodology, *Int. J. Pharm. Pharm. Sci.* 4 (2012) 550–558.
- [29] P. Venkatesan, V.S. Janardhanan, C. Muralidharan, K. Valliappan, Improved HPLC method with the aid of chemometric strategy: determination of loxoprofen in pharmaceutical formulation, *Acta Chim. Slov.* 59 (2012) 242–248.
- [30] L.H. Keith, L.U. Gron, J.L. Young, Green analytical methodologies, *Chem. Rev.* 107 (2007) 2695–2708 <https://doi.org/10.1021/cr068359e>.
- [31] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale for assessing the greenness of analytical procedures, *TrAC Trends Anal. Chem.* 37 (2012) 61–72 <https://doi.org/10.1016/j.trac.2012.03.013>.
- [32] J. Płotka-Wasyłka, A new tool for the evaluation of the analytical procedure: green analytical procedure index, *Talanta* 181 (2018) 204–209 <https://doi.org/10.1016/j.talanta.2018.01.013>.
- [33] M. Tobiszewski, J. Namieśnik, Scoring of solvents used in analytical laboratories by their toxicological and exposure hazards, *Ecotoxicol. Environ. Saf.* 120 (2015) 169–173 <https://doi.org/10.1016/j.ecoenv.2015.05.043>.
- [34] F.P. Byrne, S. Jin, G. Paggiola, T.H. Petchey, J.H. Clark, T.J. Farmer, A.J. Hunt, C.R. McElroy, J. Sherwood, Tools and techniques for solvent selection: green solvent selection guides, *Sustain. Chem. Process.* 4 (2016) 7 <https://doi.org/10.1186/s40508-016-0051-z>.
- [35] D. Prat, J. Hayler, A. Wells, A survey of solvent selection guides, *Green Chem.* 16 (2014) 4546–4551 <https://doi.org/10.1039/C4GC01149J>.
- [36] P. Bigus, J. Namieśnik, M. Tobiszewski, Implementation of multicriteria decision analysis in design of experiment for dispersive liquid-liquid microextraction optimization for chlorophenols determination, *J. Chromatogr. A* 1553 (2018) 25–31 <https://doi.org/10.1016/j.chroma.2018.04.018>.
- [37] Y. Gaber, U. Törnvall, M.A. Kumar, M.A. Amin, R. Hatti-Kaul, HPLC-EAT (Environmental Assessment Tool): a tool for profiling safety, health and environmental impacts of liquid chromatography methods, *Green Chem.* 13 (8) (2011) 2021–2025, <https://doi.org/10.1039/C0GC00667J>.
- [38] M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, ... P. Ferguson, Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator, *Green Chem.* 21 (7) (2019) 1816–1826, <https://doi.org/10.1039/C8GC03875A>.