Mestrelab Research

From instruments and analytical data to knowledge and decisions

Application Note

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Scoring of LC separation procedures for ezetimibe and some of its degradants using Mgears Chrom Best Method

Screening sample sets are common tasks in industries with discovery departments, and purification and scaling fabrication processes for high added value chemicals. In all these fields, preparative chromatography is often the preferred way to obtain material with sufficient purity. The efficient purification methodology involves screening a few chromatography methods using mass and/or UV detectors and testing different columns and/or mobile phase compositions. Frequently, these sample sets consider chiral compounds, and the objective is not only the separation of the target species such that it is as pure as possible, but also the separation of enantiomers and/or diasteroisomeric impurities. From this screen, critical decisions are taken (e.g., a synthetic route is preferred; a preparative method is chosen; alternative separation methods are tested; ...).

Frequently, a busy discovery and/or purification lab receives many such requests and reviewing the results of screens manually is time-consuming and tedious. A tool which automates the task, gathering the required data, scoring the methods for suitability, and reporting the results, is therefore highly desirable.

Mgears Chrom Best Method has been developed as a tool to hierarchically organize the results of screening experiments according to flexible, convenient, automated, and user-selectable scoring criteria. In this short note, we will consider, as a case study, the selection of LC procedures to isolate ezetimibe ($C_{24}H_{21}F_2NO_3$; CAS: 163222-33-1, MW: 409.433 g/mol) from several of its degradants.

Degradation processes of ezetimibe have been extensively described in the literature[1,2]. The sample mixture was prepared departing from an ezetimibe standard (Acesys Pharmatech, Cat No. 1242-111-21, Fairfield, NJ, USA). This standard was submitted to alkaline and acidic degradation processes, then mixed and spiked with the pure standard to provide a mixture where both the API and several of its degradants can be accurately detected. This mixture was diluted and used for all screening runs described, which were arranged according to a simple factorial experimental design (Table 1).

The column was a Cortecs C8 3 x 50 mm, 2.7 um (Waters® PN.186008369). The instrumental system used was a Waters® Acquity HClass LC system furnished with an Acquity DAD-UV detector and a Xevo TQD detector and was operated via the Masslynx software. MSD scan mode signal in the 100-700 m/z range was registered in negative polarity. The DAD wavelength range was 220-400 nm. The mobile phase flowrate was 0.2 mL/min in all experiments. The pH of the mobile phases was varied between 3 and 8, developing elutions with acetonitrile as modifier, at column temperatures of between 35 and 55 ºC.

Table 1. Experimental design in the screening study.

Run	Gradient time (min)	Temperature (ºC)	pH
1	15	35	3
2	45	35	3
3	15	45	3
4	45	45	3
5	15	55	3
6	45	55	3
7	15	35	8
8	45	35	8
9	15	45	8
10	45	45	8
11	15	55	8
12	45	55	8

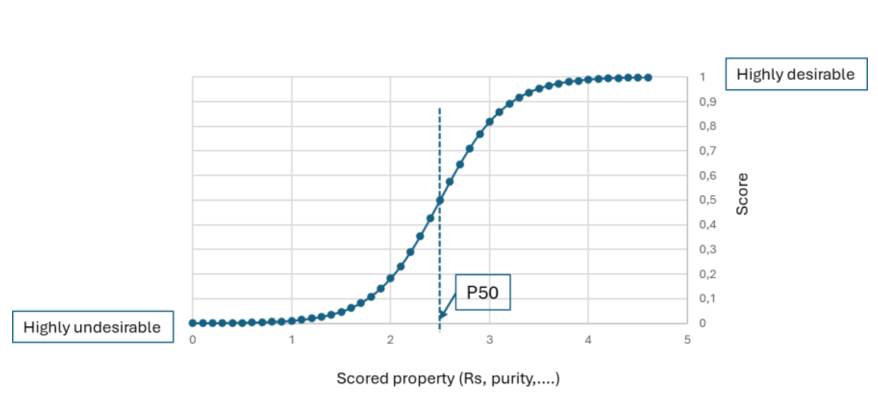
Chrom Best method scoring criteria

Chrom Best Method provides a highly flexible scoring system that can be adapted to the needs of any chromatographic separation aimed at selecting the optimal purification method. Factors such as the target resolution, purity, symmetry, and retention time can be evaluated by means of desirability functions and combined into an overall measure, allowing the selection of the best separation among those provided in the experiment.

[1] Stability indicating RP-HPLC method for simultaneous determination of simvastatin and ezetimibe from tablet dosage form, *R.P. Dixit, C.R. Barhate, S.G. Padhye, C.L. Viswanathan, M.S. Nagarsenker, Ind. Jour. Pham. Sci. 2010, 72(2) 204-210*.

[2] Development and validation of a novel stability indicating HPLC method for the quantitative determination of eleven related substances in ezetimibe drug substance and drug product. *Zhiqiang Luo, Zhongqing Deng, Yang Liu, Guopeng Wang, Wenning Yang, Chengbo Hou, Minming Tang, Riurui Yang, Huaming Zhou*, Talanta, 2015, 139, 67-74.

Desirability functions for scoring criteria use logistic functions (Figure 1) that represent situations that range from highly undesirable (score = 0) to highly desirable (score = 1). These functions are defined by assigning a value for the 50% score point and a slope (k). Moreover, a weight coefficient can be assigned to reflect the relative importance of each scoring criterion to the user.



$$Score = \frac{L}{1 + e^{-k(x - P_{50})}}$$

L = weight

K = slope

P50 = value giving 50% of max score

Figure 1. Desirability functions used by Chrom Best Method

Some of these functions are quite straightforward, such as those for peak purity, symmetry, and retention time. For example, the purity criterion is defined by fixing the purity level for P50 (e.g., 95%) and the slope. This allows for more or less demanding conditions for separation. For example, in Figure 2 we see how the purity criterion can be relaxed, changing the slope value even though the P50 condition remains the same. Similar behavior may be found in other unilateral desirability criteria (e.g., resolution and similarity), and even in the retention time criterion, although in this case the desirability function defined is a bilateral one.

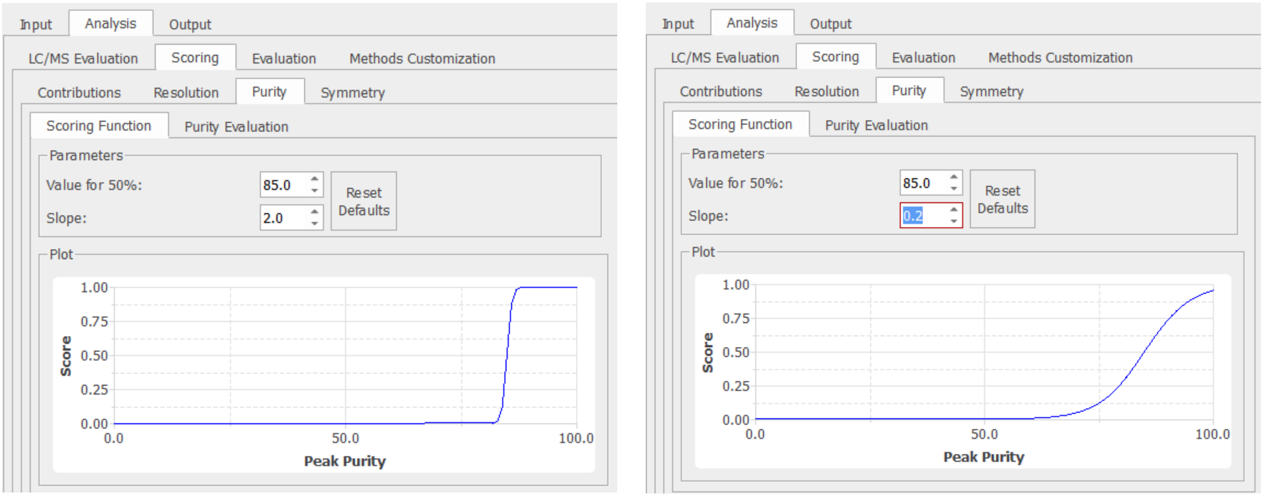


Figure 2. Controlling the target peak purity criterion.

Resolution criterion is a bit more complex because, to allow maximal flexibility, critical values are defined for peaks eluting before and/or after the target peak. Moreover, the basic Rs criterion is combined with another term that considers the maximum percentage of impurity allowable. In this way, the criterion can be controlled and adapted to virtually any practical situation.

In the instance of our case study experiments (Table 1), the scoring criteria we configured are as detailed in Table 2. Overall scores were calculated using the geometric mean of individual criteria scores. This retains the normalized scale (0-1) for the final scores, thus making the comparison between runs very easy.

Rs score	Purity score (*)	Symmetry score	Rt score
• P50 before target: 2.0 • P50 after target: 2.0 • Rs Slope: 1.5 • Impurity fraction (P50): 0.10 • Imp. Fraction slope: 45.0	• P50: 85.0 • Slope: 0.4 • Inter-method filter: off • Intra-peak filter options: ○ Masses larger than target (except adducts) are impurities. ○ Masses smaller than target are fragments (if Rt matches).	• P50: 0.7 • Slope: 10.0	Not used

Table 2. Settings defined for scoring the 12 chromatographic runs in Table 1. (*) Purity scoring applies the powerful matching options provided by Mnova for MS signals and, optionally, the inter-method filter[3] to provide enhanced reliability in MS signals processing)

Chrom Best Method results

Enter the path of the raw data files for experiments and launch the Chrom Best Method. A summary of information about the runs' scoring will be produced in the Mgears viewer (Figure 3). The color codes associated to calculated overall scores (from green to red on decreasing the overall score) gives immediate insight about the adequacy of any of the runs to our separation goals.

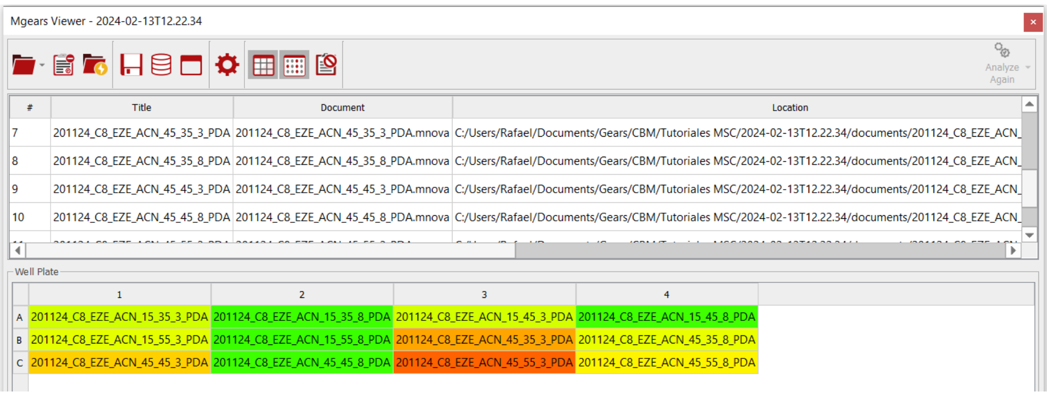


Figure 3. Mgears viewer summarizing the scoring results as produced by Chrom Best Method using the settings reported in Table 2.

By clicking on any of the table rows (or in any of the cells in the well plate), a full detailed set of results for the corresponding run will be offered (Figure 4). Here, we can appreciate the details of the separation obtained

[3] See Mgears Chrom Best Method documentation for a detailed description of these options.

and assess the scores generated, making decision-taking easy. If the option was selected on configuring Chrom Best Method, a pdf document will be generated for each run that includes all that information.

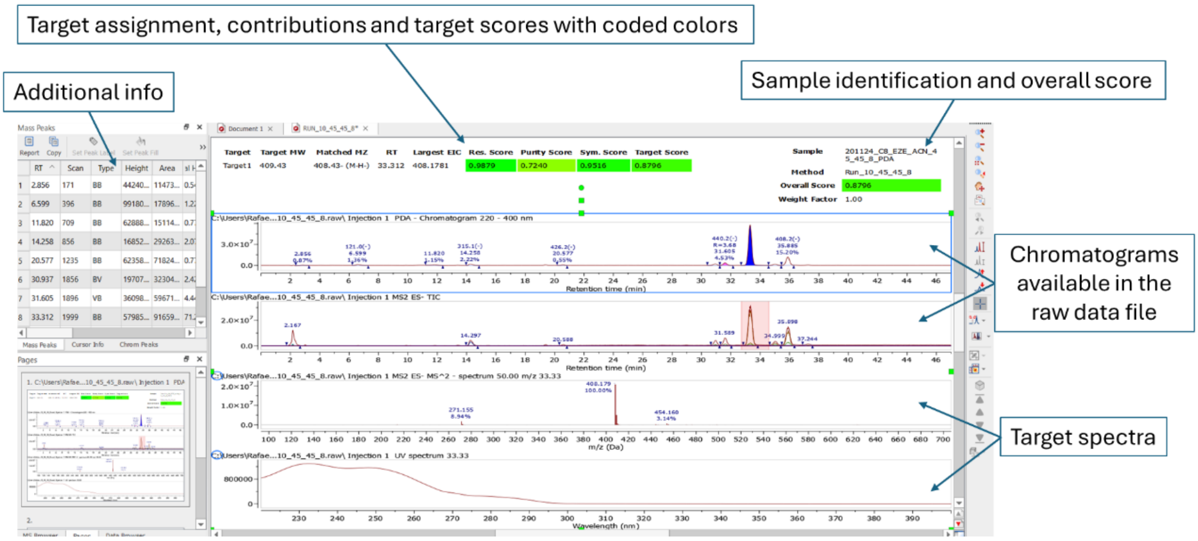
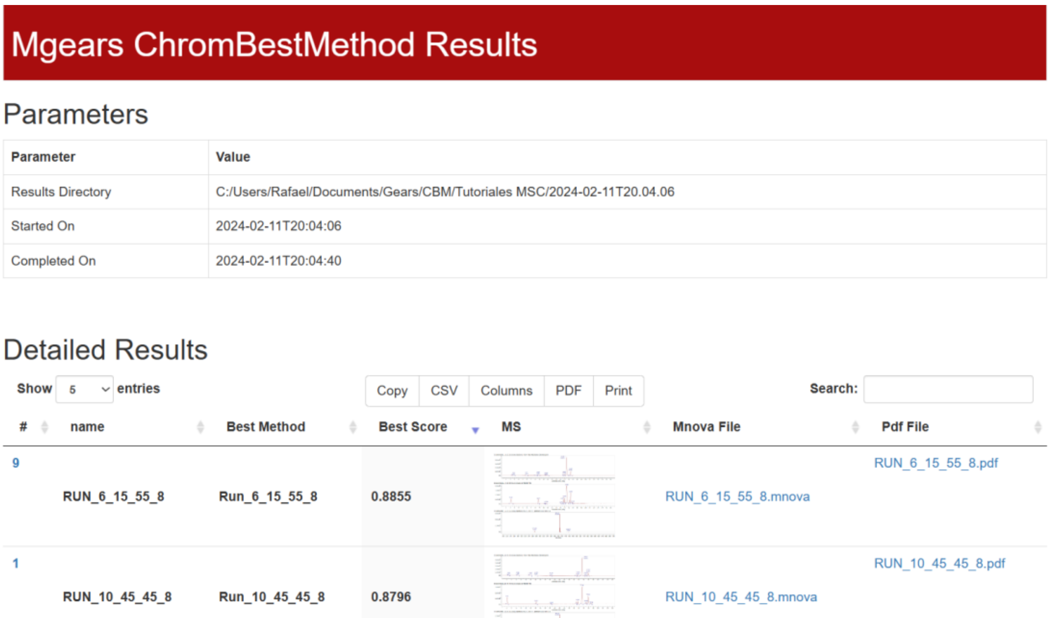


Figure 4. Results for Run 10, as shown in the Mnova document viewer.

Although run results can be viewed run-after-run, a summary of results with links to all available data can be found in the ChromBestMethod-Results.html, (Figure 5), which can be found within the output directory. These results are very easy to manage because columns in the table can be ordered to allow precise comparisons. For example, in Figure 5, we have ordered the information by the Best Score column so we see that run 6 obtained the highest score in our experiment (0.8855), but we can also see that some other runs obtained scores that were quite similar. For example, run 10 obtained a score of 0.8796 but required about 34 minutes to elute the target (Figure 4), whereas run 6 needed less than 16 minutes to elute the target (Figure 6), so run 6 would clearly be advantageous.



Mgears ChromBestMethod Results

Parameters

Parameter	Value
Results Directory	C:/Users/Rafael/Documents/Gears/CBM/Tutoriales MSC/2024-02-11T20.04.06
Started On	2024-02-11T20:04:06
Completed On	2024-02-11T20:04:40

Detailed Results

Show 5 entries — Copy | CSV | Columns | PDF | Print — Search:

#	name	Best Method	Best Score	MS	Mnova File	Pdf File
9	RUN_6_15_55_8	Run_6_15_55_8	0.8855		RUN_6_15_55_8.mnova	RUN_6_15_55_8.pdf
1	RUN_10_45_45_8	Run_10_45_45_8	0.8796		RUN_10_45_45_8.mnova	RUN_10_45_45_8.pdf

Figure 5. Summarized results as viewed in the ChromBestMethod-Results.html file. Runs have been ordered by the Best Score column.

The Mgears Chrom Best Method plugin for Mnova provides a powerful tool to help the experimenter take accurate and rapid decisions about the more convenient elution procedure in purification and up-scaling processes based on multiple criteria. Since the scoring process is fully automated and, at the same time, sufficiently flexible to accept user expertise, valuable time and accuracy are gained while minimizing the need for manual review.

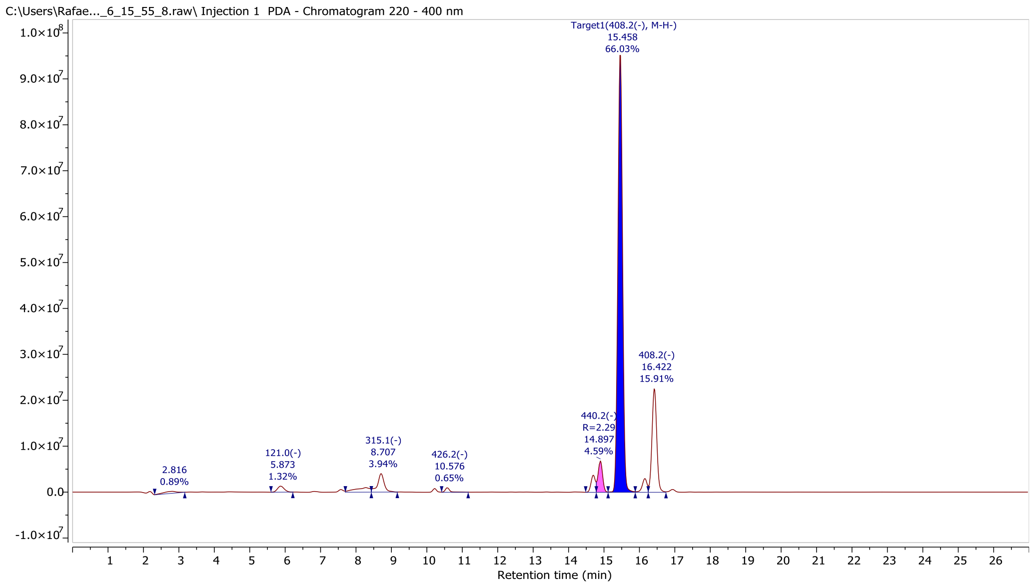


Figure 6. Total absorbance chromatogram of run 6.

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