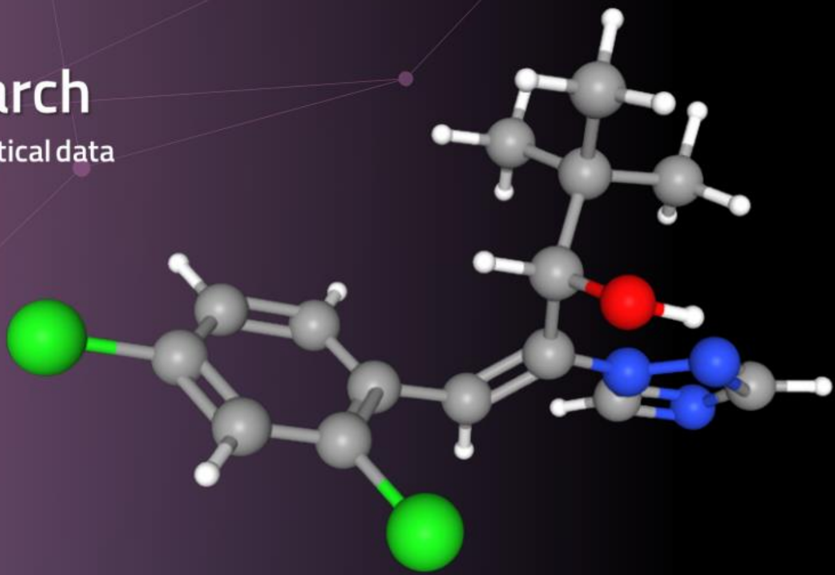
Mestrelab Research
From instruments and analytical data
to knowledge and decisions



Application Note

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Scoring of LC separation procedures for a chiral compound using Mgears Chrom Best Method

Screening sample sets is a frequent task in industries with discovery departments, alongside purification and scaling of fabrication processes for high-value chemicals. In these areas, preparative chromatography is often the favored method for achieving materials with adequate purity. Efficient purification methodology involves screening a few chromatographic methods via MS and/or UV detectors and testing different columns and/or mobile phase compositions. Such sample sets frequently consider chiral compounds, where 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. Critical decisions are commonly highly dependent on such screening (e.g., a particular synthetic route is preferred; a preparative method is chosen; alternative separation methods are tested; ….).

Discovery and purification labs frequently handle numerous such requests, making the manual review of screening results both time-consuming and tedious. Thus, a tool that automates this task by collecting the required data, evaluating the methods for suitability, and reporting the results is highly valuable.

Mgears Chrom Best Method has been designed to hierarchically organize the results of screening experiments using flexible, convenient, automated, and user-selectable scoring criteria. In this brief note, we will present a simple case study focused on chiral targets, specifically the selection of LC procedures to separate diniconazole ($C_{15}H_{17}Cl_2N_3O$; CAS: 83657-24-3, MW (monoisotopic): 325.07 g/mol). A second Application Note in this series will also address the separation of this compound and several of its chiral UV degradation products.

Sample preparation

The sample was prepared from a diniconazole standard (Riedel de Haen Pestanal Cat No. 70217-36-6; Merck KGaA, Darmstadt, Germany) which is supplied as a racemate of Z and E isomers.

The column was an Acquity® UPC Trefoil Cel-2 (50 x 3.0 mm, 2.5 µm) (Waters® PN.186007656), The instrumental system used was a Waters® Acquity HClass LC system furnished with an Acquity DAD-UV detector and a Xevo TQD detector, operated via the Masslynx software. An MSD scan mode signal in the 50-650 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. Table 1 reports the experimental design using isocratic elutions with acetonitrile as the modifier.

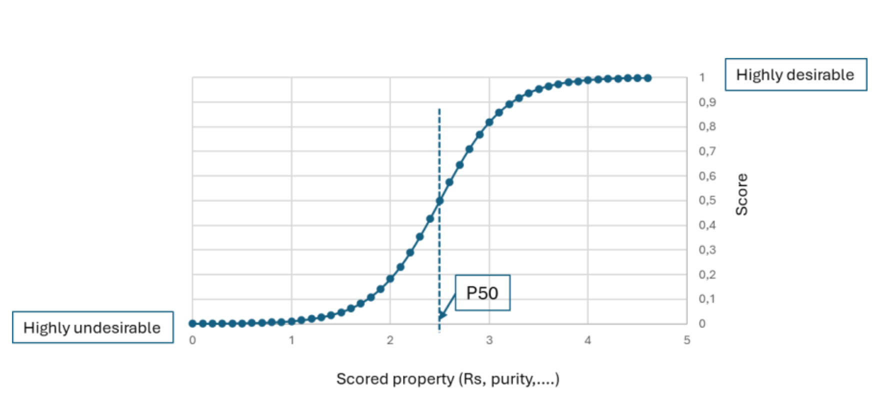
Table 1. Experimental design in the screening study.

Run	% modifier	Temperature (ºC)
1	60	30
2	60	40
3	50	30
4	50	40
5	40	30
6	40	40
7	30	30
8	30	40

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 an 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 for the selection of the best separation among those provided in the experiment.

Desirability functions for scoring criteria use logistic functions (Figure 1) that represent circumstances that range from highly undesirable (score = 0) to highly desirable (score = 1). These functions are defined by assigning a value to the 50% score point and a gradient (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 gradient. This allows the flexibility for more or less demanding conditions for separation. For example, in Figure 2 we can see how the purity criterion can be relaxed, changing the gradient 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.

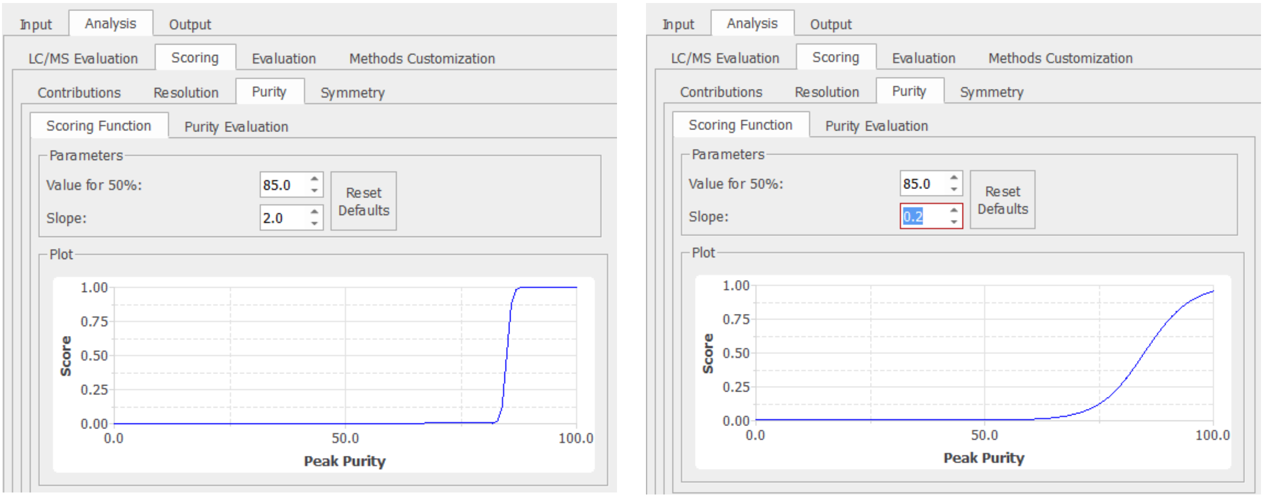


Figure 2. Controlling the target peak purity criterion.

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 allowable percentage impurity. In this way, the criterion can be controlled and adapted to virtually any practical situation.

Finally, the information about the chirality and the number of chiral centers should be provided to allow the Chrom Best Method plugin to adapt its behavior to chiral targets. Although the number of targets in Chrom Best Method can be freely defined, for chiral targets the system is prepared to identify and score peaks exhibiting the same spectral behavior as a function of the number of chiral centers in the target molecules.

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 straightforward.

Rs score	**Purity score (*)**	**Symmetry score**	**Rt score**	**Additional target specifications**
• P50 before target: 1.5 • P50 after target: 1.5 • Rs Slope: 1.5 • Impurity fraction (P50): 0.10 • Imp. Fraction slope: 45.0	• P50: 85.0 • Slope: 0.1 • Inter-method filter: on • 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	• Chiral = yes • Number of chiral centers =1 • Relative EIC peak height % threshold = 10.0

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

Chrom Best Method results

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

By clicking on any of the table's rows (or in any of the cells in the well plate), a fully detailed set of results for the corresponding run will be offered (Figure 4). Here, we can appreciate the details of the separation obtained and assess the scores generated, making decision-taking easy. If the appropriate option was selected on configuring Chrom Best Method, a pdf document will be generated for each run that includes all this information.

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

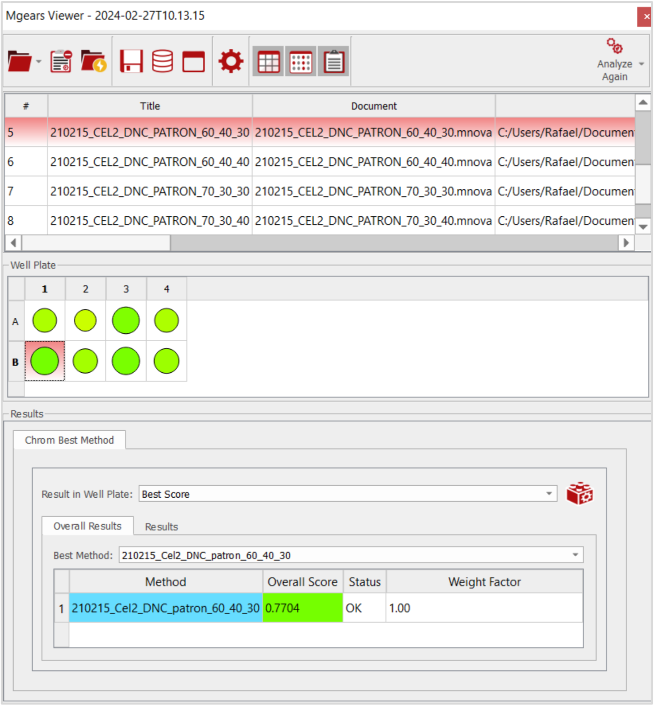


Figure 3. Mgears viewer summarizing the scoring results as produced by Chrom Best Method using the settings reported in Table 2.

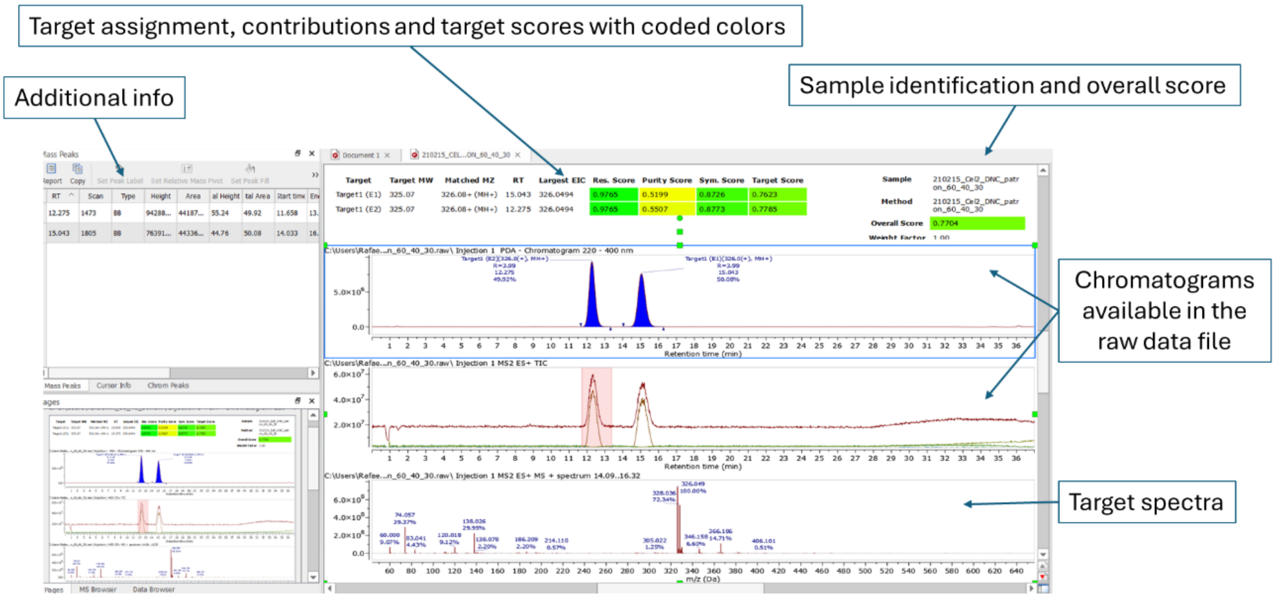
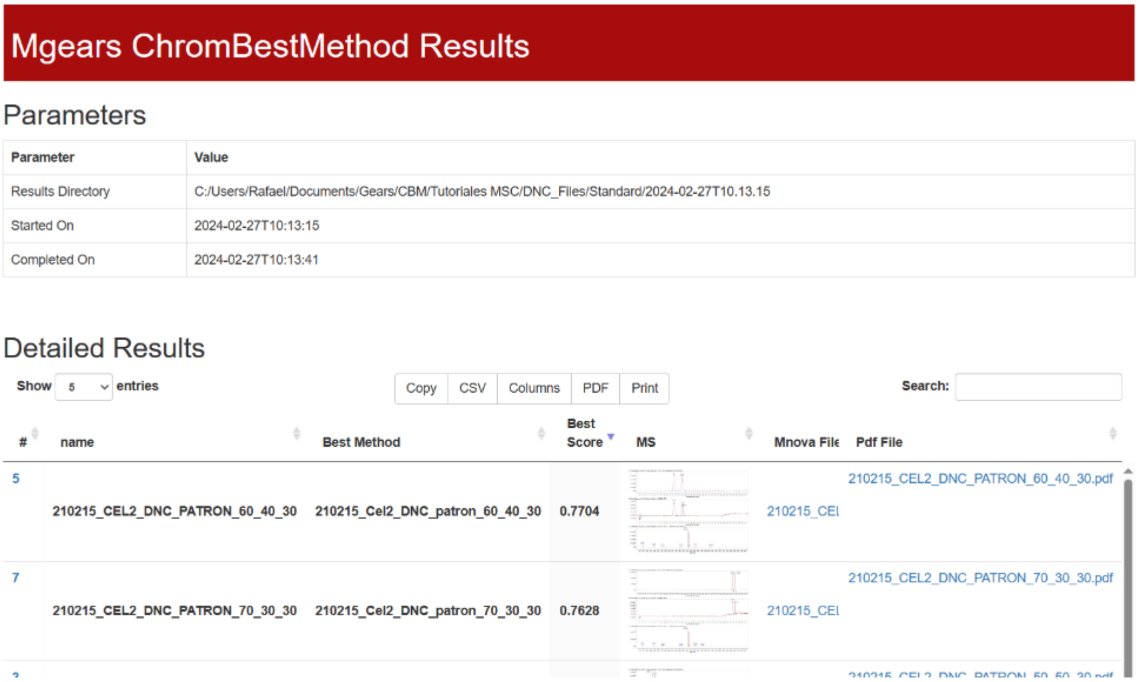


Figure 4. Results for Run 5 as shown in the Mnova document viewer.

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 file, (Figure 5), which can be found within the output directory. These results are extremely 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 5 obtained the highest score in our experiment (0.7704); however, we can also see that some other runs obtained similar scores (runs 3 and 7). Run 7, for instance, obtained a score of 0.7628 but required about 33 minutes to elute both isomers, whereas run 3 required less than 7 minutes to complete the elution and has a score of 0.7485, so run 3 would be clearly advantageous (Figure 6).



Mgears ChromBestMethod Results

Parameters

Parameter	Value
Results Directory	C:/Users/Rafael/Documents/Gears/CBM/Tutoriales MSC/DNC_Files/Standard/2024-02-27T10.13.15
Started On	2024-02-27T10:13:15
Completed On	2024-02-27T10:13:41

Detailed Results

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

#	name	Best Method	Best Score	MS	Mnova File	Pdf File
5	210215_CEL2_DNC_PATRON_60_40_30	210215_Cel2_DNC_patron_60_40_30	0.7704		210215_CEI	210215_CEL2_DNC_PATRON_60_40_30.pdf
7	210215_CEL2_DNC_PATRON_70_30_30	210215_Cel2_DNC_patron_70_30_30	0.7628		210215_CEI	210215_CEL2_DNC_PATRON_70_30_30.pdf

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

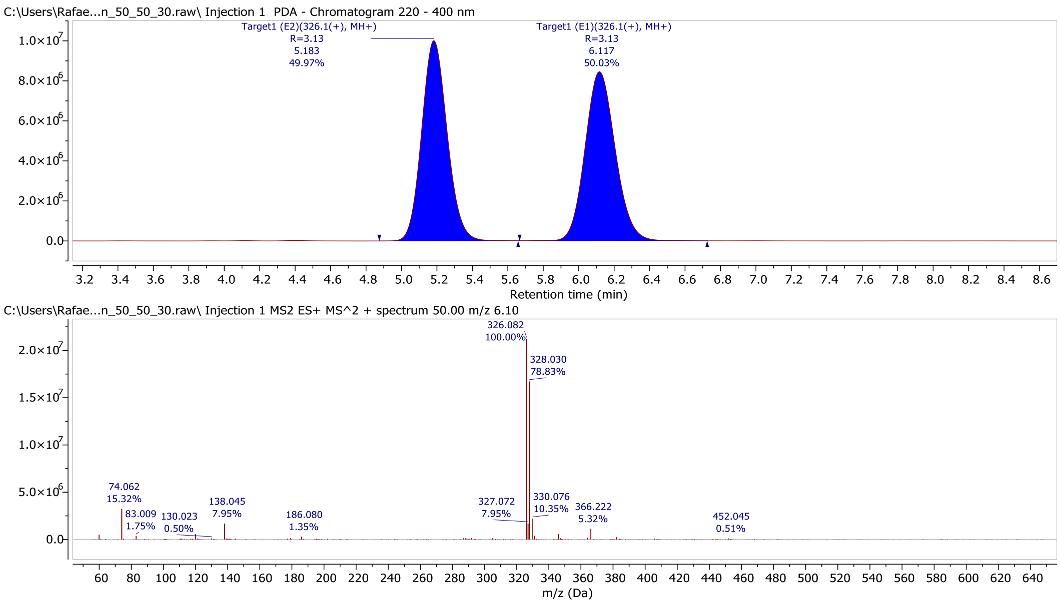


Figure 6. Total absorbance chromatogram and MS spectrum for run 3.

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

Acknowledgements.

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Learn more about Chrom Best Method at
mestrelab.com/software/Mnova-gears-chrom-best-method/

Contact us for general information or sales inquiries.