Assessing parallelism using Parallel-Line Analysis in MARS

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- **Parallel-line analysis is applied to inhibitor binding data of a Prostaglandin D Synthase (hematopoietic-type)**
- **Fluorescence Polarization-based inhibitor screening assay estimates relative binding potencies**
- **CLARIOstar®Plus performs FP-measurement and PLA analysis**

Introduction

Screening for drug compounds with higher potency is of keen interest for many pharmaceutical drug development departments. Finding compounds which lead to the same response as a reference substance but at a lower dose can be the key to lower the risk of side-effects and to lower manufacturing costs. These types of bioassays, however, are also very common in other research areas. Parallel-line analysis (PLA) is often applied in this context in order to prove parallelism of the underlying dose-response curves and subsequently estimate the relative potencies of substances compared to a reference substance. Here we describe how to apply PLA in BMG LABTECHs data analysis software MARS to inhibitor binding data to assess binding potencies of Prostaglandin D synthase inhibitors.

Principle of PLA

Parallel-line assays are common in drug efficacy testing wherein one or more test substances are compared against a reference substance based on their relative potency. The latter shall only be calculated if the underlying dose-response curves are parallel. Parallel-line analysis (PLA) is the statistical way to assess if curves are parallel, and if so, calculates the relative potencies of the substances.

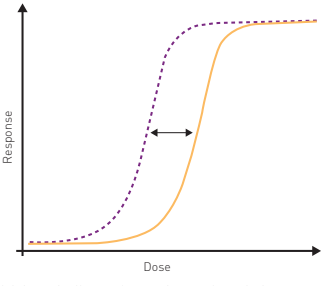

Fig. 1: PLA is typically used to estimate the relative potency (black arrow) of a test substance (orange) compared to a reference substance (purple).

Fig. 1 shows two typical dose response curves of a test (purple) and a reference substance (orange); both having comparable slopes and asymptotes thus considered parallel.
PLA can be done using different statistical approaches on the basis of these parameters to assess parallelism of the curves.

Difference testing to assess parallelism

Using a difference testing approach, the test and reference curves are fitted with shared parameters (constrained fit, global fit). At the same time all curves are fitted with no parameters shared among the different curves (unconstrained fit). The Residual sum of squares of these fits are compared based on an F- or Chi²-Test, respectively. A p-value is calculated indicating if the unconstrained (p-value is much smaller than 1) or the constrained model is preferred (p-value is close to 1). This concept is also known from analysis of variance (ANOVA) giving identical final results.

Equivalence testing to assess parallelism

While the aforementioned difference testing approach compares reference and test curves based on a fit quality metric, the equivalence testing approach compares the fit parameters itself. Therefore, MARS fits the test curves and reference curve individually and subsequently calculates the ratios of the corresponding fit parameters (e.g. slope and asymptotes) for each reference and test curve pair. Confidence intervals for these ratios are additionally calculated according to Fieller's theorem. A given reference and test curve can be considered parallel if the calculated confidence intervals fall completely within a pre-defined equivalence interval. The equivalence interval is either based on historical data, on reference data or given by some legal agency

PLA of inhibitor binding data using MARS

We applied PLA to data of a Prostaglandin D Synthase Inhibitor (PGDS) Screening Assay (Cayman Chemicals #600007) wherein two compounds, the novel inhibitor TFC 007 and the well-characterized reference inhibitor HQL-79, were applied in various concentrations to replace a pre-bound and fluorescently labeled PGDS inhibitor. Binding was followed by changes in fluorescence polarization detected by the CLARIOstar Plus reader.

Instrument settings

Optic settings	Fluorescence polarization, end-point	
	Filters	482-16/ LP504/530-40
	Gain and focus	Adjusted prior to measurement
	Target mP	200 (on DMSO control)
General settings	Flash number	200
	Settling time	0.1 s

*For details regarding instrument settings and assay background information please refer to BMG LABTECH Application Note 285.

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The binding curves were analyzed to estimate the relative (binding) potency of TFC 007 compared to HQL-79. To this end the MARS analysis software that comes with the reader was employed. The Parallel-line analysis window is accessible via the Calculations button in the Home tab. Please note that PLA in MARS requires the presence of standards with at least two groups in the microplate layout with corresponding concentration or dilution values. One group needs to be defined as the reference group and the other groups correspond to the data of the test compounds. One can either choose to compare only selected test compound groups with the reference group or compare the reference groups to all other compound groups. MARS offers different models to fit the corresponding curves. Testing for parallelism of the curves can either be done by difference testing or by equivalence testing (see Fig. 2). In the former case one has to choose the parameters that will be shared during the fit procedure while in the latter case these parameters will be used for the ratio calculation mentioned in the previous section.

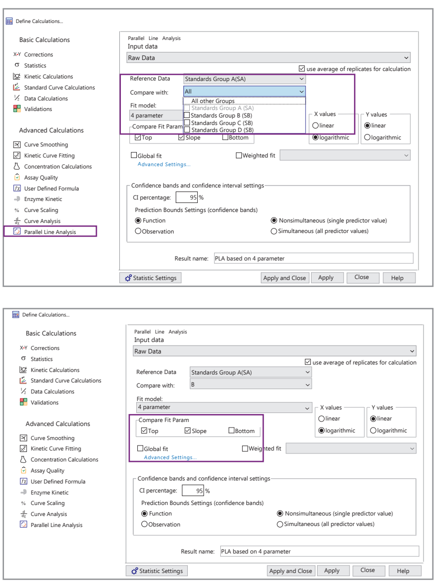
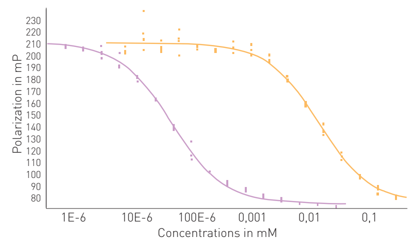


Fig. 2: (Top) The presence of grouped standards as well as concentrations defined in the microplate layout are required to perform PLA in MARS. Among other things the reference data and test data need to be defined in the PLA window. (Bottom) Either an equivalence testing approach (Global fit unchecked) or a difference testing approach (Global fit checked) can be used to assess parallelism.

Results & Discussion

PLA in MARS was used to estimate the inhibition potency of TFC 007 compared to HQL-79. PLA reveals a log(relative potency) of about 2.46 of TFC 007 compared to HQL-79 which corresponds to a relative potency of about 300 (see Fig. 3). This means that TFC 007 has a 300-fold higher inhibition potency than HQL-79. Please note that the log(relative potency) can be transformed to the relative potency in MARS by taking this fit parameter to the power of 10.



All curves can be considered parallel according to a F-test

Group: A(HQL-79) (Ref.)

Parameter	Value	95% CI min	95% CI max
Top	204,4	202,3	206,5
Slope	-1,0007281	-1,0826432	-0,9188130
EC50	0,013	0,012	0,015
log(EC50)	-1,884	-1,932	-1,836
Bottom	75,3	72,8	77,9
r	0,9976266	-	-
r^2	0,9952589	-	-
Curve Color:			

Group: B(TFC 007)

Parameter	Value	95% CI min	95% CI max
Top	204,4	202,3	206,5
Slope	-1,0007281	-1,0826432	-0,9188130
EC50	45,444E-6	40,648E-6	50,806E-6
log(EC50)	-4,343	-4,391	-4,294
Bottom	75,3	72,8	77,9
Parallel	Yes	-	-
log(rel. potency	2,4581360	2,4034287	2,5128433
r	0,9994554	-	-
r^2	0,9989111	-	-
Curve Color:			

Fig. 3: Global fits of TFC 007 (purple) and HQL-79 (yellow) at the top and the corresponding fit results at the bottom.

Conclusion

Parallel-line analysis (PLA) is a common approach to assess parallelism of dose-response curves which are often obtained in biological assays. Different statistical approaches were implemented into BMG LABTECH's data analysis software MARS in order to assess dose-response curve parallelism and to estimate relative potencies. Here the (relative) binding potency of the hematopoietic prostaglandin D synthase inhibitor TFC 007 was estimated to be about 300-fold higher than that of HQL-79 using PLA in MARS.

References

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