Supporting Information for Robustness in the fitting of Molecular Mechanics parameters

Kenno Vanommeslaeghe*[1], Mingjun Yang[1], and Alexander D. MacKerell Jr.†[1]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland 21201

Contents

S1 Introduction

S1.1 Optimization problems

In the general case, an optimization involves finding values for n parameters such that a merit function that depends on these parameters attains an optimal value. This simple premise has spawned a subfield of computer science and mathematics. Listing all the optimization algorithms that are currently available goes far beyond the scope of this short introduction, but it bears mention that different classes of optimization problems require different optimization algorithms. Specifically, if the merit function is unknown or not analytically solvable, the optimization is a trial-and-error process. If the merit function has multiple minima, it is often desirable for these trials to explore parameter space, as opposed to finding a single local minimum close to a given starting point. Depending on known or assumed properties of the merit function, trials will be based on extrapolation from earlier trials; for example, a common assumption is "approximately quadratic as a function of the parameters". If first derivatives of the merit function are available, this extrapolation can be made substantially more accurate, especially if the dimensionality of the problem (ie. the number of independent parameters) is high. Second derivatives in theory pose an opportunity to refine the extrapolation even further, although in practice, they are often (but not always) impractical on account of being too costly to calculate and/or too unwieldy (there are n^2 second derivatives) and/or do not sufficiently improve the extrapolation; indeed, if the merit function is far from quadratic, second derivatives can cause erratic behavior.

S1.2 Multi-objective optimization

A generalization of the above problem is the multi-objective optimization problem, where the goal is to find values for n parameters such that m numerical properties (henceforward called "observables") that are dependent on said parameters each approach respective target values. Both the parameters and the properties may be subject to constraints and restraints, some of which will be discussed in more detail. Optimization problems in this class can formally be expressed as a system of equations, where the parameters to be optimized are the variables, the left hand side of the equations expresses how the observables depend on the variables, and the right hand side consists of the respective target values. As in the previous paragraph, the equations themselves are not guaranteed to be linear, analytically solvable, or even known. If the equations are analytically solvable, the optimization problem is nontrivial only if the system of equations is mathematically inconsistent, i.e. an exact solution does not exist. Thus, the objective is to find one or more approximate solutions for which the vector of observables approaches the vector of target values. However, in contrast to the scalar merit functions in the previous paragraph, there does not exist a "perfect" criterion to rank how well vectors approach a target; hence, multi-objective optimization problems may have different end-goals and associated solution philosophies depending on the context in which they arise. Examples of common end-goals include obtaining a set of Pareto optimal solutions, or quantifying the trade-offs in satisfying the different objectives. Another possibility is to express the "fitness" of an approx-

---

*To whom correspondence should be addressed: kvanomme@rx.umaryland.edu

†To whom correspondence should be addressed: amackere@rx.umaryland.edu

imate solution as a simple scalar numeric value that is a function of the observables and their target values. Introducing a single merit function in this fashion reduces the problem to the single-objective case discussed in the previous paragraph.

S1.3 The RESP Model

The desire for robustness in fitting problems is not new; a prominent example in the field of Molecular Mechanics is electrostatic potential-based charge fitting, where the parameters are point charges on the atoms in a molecule, the target vector consists of electrostatic potentials measured at different positions around the molecule, and the coefficients in the linear system are derived from the fixed positions of the aforementioned point charges and measurement points through Coulomb's law. Lack of robustness because of ill-conditionedness is commonly observed when calculating an LLS solution to this problem, which led Bayly *et al.* to propose adding restraints to the system.[1] Specifically, the preferred variant of their RESP method includes a constant restraint that pulls every charge towards 0. The most important limitation of this methodology is that the restraint needs to be small enough to avoid pulling down well-defined charges too strongly, but large enough to overcome noise in the system. This not only includes numerical noise caused by rounding errors, but also features of the Quantum Mechanics (QM) electron density the Electrostatic Potential (ESP) of which cannot be reproduced by atom-centered point charges. Indeed, in this situation, the LSS algorithm is prone to overfit, i.e. assign unphysical charges on poorly defined (typically buried) atoms in an attempt to reproduce these features. The compromise restraint Bayly *et al.* obtained through trial and error suffers to a small extent from both problems, which were mitigated as follows:

- The small restraint-induced decrease in magnitude of well-determined charges turned out to be generally desirable, as the chosen QM level of theory (HF/6-31G*) is known to overpolarize most molecules to a much larger extent than the effect of the restraint.

- Harmonic restraints have mathematically convenient properties and are trivial to implement in a LLS context (see section 2.6), but they penalize large charges stronger than small ones, resulting in bigger restraint-induced absolute errors on polar groups. This turned out to be an significant issue for charge fitting, and was overcome by using a hyperbolic restraint instead, at the (small) cost of the problem becoming nonlinear and requiring iterative solving.

- The limitations of the atom-centered point charges in describing the QM ESP turned out to be a relatively benign as such, except when the freedom of the fit was further constrained by requiring chemically equivalent atoms to have the same charge, which resulted in a large discrepancy between the asymmetric QM description and the partially symmetrized point charges. This required the *ad hoc* workaround of first performing an unconstrained fit, after which the resulting charges on all atoms are frozen, except the chemically equivalent atoms, which are forced to be equivalent, and their parent atoms.

Since the publication of Bayly *et al.*'s seminal paper, the above workarounds have proven highly successful, attaining the stated goal of making the derivation of point charges from an ESP robust enough to be "a general and useful way to generate atomic charges for simulations of complex systems". Nevertheless, it should be noted that these same workarounds are highly specific for the problem of fitting atom-centered point charges to HF/6-31G* ESP for the purpose of molecular mechanics simulations in the presence of the explicit TIP3P or SPC water models.

S2 Methodology

S2.1 Sources of ill-conditionedness in bonded parameter fitting

S2.1.1 Symmetry-forbidden multiplicities

The simplest case of ill-conditionedness in bonded parameter fitting is the one where two identical dihedrals around the same bond cancel each other out. Take for example the rotation of a carboxylate group or benzene ring attached to a larger molecule. The dihedral angle governing this rotation involves a trigonal planar atom (the *sp*^{2} carbon) with two identical substituents (the carboxylate oxygen or *ortho* benzene carbon atoms). This leads to two identical dihedral terms with an offset of 180° in their ϕ angles. It can easily be shown that these terms mathematically cancel out for odd values of n_i; this is a commonly made mistake when selecting n_i values to be fitted with existing automatic fitting programs. Similarly, terms associated with n_i values that are not a multiple of 3 will mathematically cancel out for rotations of tetrahedral atoms with 3 identical substituents, where two of the ϕ angles are offset 120°and –120°, respectively, with respect to the first.[*] Examples are the rotations of trifluoromethyl, sulfonate and quaternary trimethylamino groups. The two cases can even occur together in one rotatable bond in molecules such as trifluoroacetate, trifluorotoluene, benzenesulfonate and the N,N,N-trimethylanilinium cation (compounds **1**, **2**, **3** and **4** in figure S1, respectively), where the only valid n_i value is 6.

---

[*] i.e. $K_i^{\phi}(1+\cos(n_i\phi)) + K_i^{\phi}(1+\cos(n_i(\phi+\pi))) = 2K_i$ for odd values of n_i and $K_i^{\phi}(1+\cos(n_i\phi)) + K_i^{\phi}(1+\cos(n_i(\phi+2\pi/3))) + K_i^{\phi}(1+\cos(n_i(\phi-2\pi/3))) = 3K_i$ for n_i values that are not multiples of 3; the constant offsets $2K_i$ and $3K_i$ can be ignored for the present purpose, as explained in section 2.1.

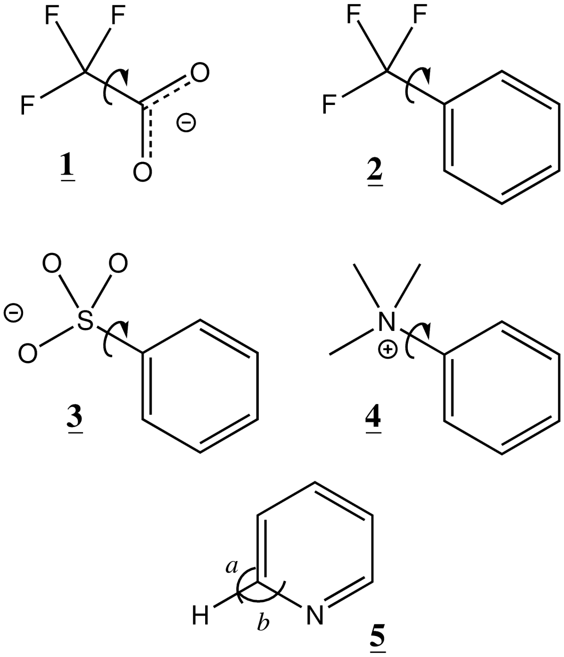

Figure S1: Skeletal formulas of compounds discussed in the Supporting Information, with representation chosen to highlight attributes of interest. **1**, **2**, **3** and **4** are respectively trifluoroacetate, trifluorotoluene, benzenesulfonate and the N,N,N-trimethylanilinium cation, with the rotatable bond that can only have a 6-fold multiplicity marked. **5**: pyridine, with the coupled angles a and b discussed in section S2.1 marked.

In the above discussion, idealized geometries were assumed (i.e. offsets in ϕ of *exactly* 180°, 120°and –120°), giving rise to a linear system that is truly underdetermined. However, forces in actual molecules almost always cause small deviations in the angles and offsets, so that a change in K_i^ϕ has a small residual impact on the observable energy profile $\mathbf{B}'$, which the algorithm will try to exploit to fit arbitrary features in the target potential energy surface $\mathbf{B}$. Just like in section 1.2, this will lead to very large K_i^ϕ values, which are extremely problematic from a transferability point of view, and may even induce distortions in the structure.

S2.1.2 Underdetermined fit of valence angles

Consider the valence angles involving the *ortho*-hydrogen atom of a pyridine ring (compound **5** in figure 1). Assuming the ring is relatively rigid and all reference angles are 120°, the force constant for the overall in-plane hydrogen deformation is simply the sum of the force constants of the 2 angle terms involving this hydrogen atom ($K_a + K_b = K_{target}$). The out-of-plane potential due to the angle terms will have a slightly more complicated functional form depending on how the out-of-plane DF is defined, but it still features the sum $K_a + K_b$, so although this is an independent DF from a *geometrical* point of view, it is not independent from the point of view of the parameters K_a and K_b. Moreover, even in the absence of an improper dihedral term, the hydrogen atom participates in three dihedral terms that influence the out-of-plane potential, exacerbating the underdetermination.

S2.1.3 Implicit and unintentional mitigation of ill-conditionedness

In an ill-conditioned system, the gradient in parameter space that differentiates the exact LLS solution from solutions for which $\mathbf{B}'$ is almost the same but the K_i^ϕ values are very different is by definition very low. Both local optimizers and MCSA are relatively insensitive to such low gradients; for the former, it may fall below the gradient tolerance, while for the latter, a step that goes against a very low gradient only marginally increases the energy and therefore has a good chance of being accepted by the Metropolis criterion. In the case of the local methods, this will result in the optimizer finding an approximate solution that is substantially closer to the initial guess than the exact solution. Conversely, in the specific case of Guvench *et al.*'s MCSA method,[2] it was observed in practical usage that different runs with different random number seeds often resulted in widely diverging parameter sets with almost identical energy profiles $\mathbf{B}'$. The common solution to this problem was to take the average of the output parameter values from several runs, and a lot of published results are based on this methodology.[3] Indeed, it trivially follows from the linear nature of the system that a linear combination of possible $\mathbf{X}$ vectors will yield the same linear combination of the corresponding $\mathbf{B}'$ vectors, i.e. $\mathbf{A}(a\mathbf{X}_a + b\mathbf{X}_b + \dots) = a\mathbf{B}'_a + b\mathbf{B}'_b + \dots$, justifying the averaging. More importantly, as the K_i^ϕ values are typically constrained between -3 and 3 kcal/mol in this methodology, the average of multiple runs with arbitrary output K_i^ϕ values will statistically favor a solution with low $\left|K_i^\phi\right|$ values, which is often desirable for dihedrals.

S2.2 Disproportional scaling of parameters by constant harmonic restraints

The observation that some parameters (the larger ones in Bayly *et al.*'s case) are scaled disproportionally by a constant harmonic restraint is a nontrivial one. If we consider an initial guess of 0, the restraining *force* is proportional to the magnitude of the parameter. However, making a larger parameter deviate from its unrestrained value by a given factor also requires a proportionally larger force. Indeed, if two parameters have orthogonal response vectors of the same magnitude, but one optimizes to a larger value, then a constant harmonic restraint will pull down both by an equal factor, so the larger parameters is only biased stronger in an absolute sense. Conversely, if the difference in the magnitude of the parameters with orthogonal response vectors is purely caused by differences in the magnitudes of the response vectors, then the larger parameter will contribute to the error function less than the smaller one, and will indeed be biased stronger even in a relative sense. Finally, the effect of constant restraints becomes nontrivial for non-orthogonal response vectors; this is discussed in detail in sections 2.6-2.10.

S2.3 Potential Energy Scanning considerations

The present algorithm's ability to perform sub-second fitting while avoiding trivial cases of ill-conditionedness gave us an unprecedented opportunity to identify more fundamental issues related to the target data. Therefore, the current section will discuss target data generation considerations that came to light when applying the present algorithm to practical systems (see also section 4.2.1). The chief concern here is orthogonality between the target data generated by different 1-dimensional potential energy scans, as higher-dimensional scans quickly become computationally prohibitive. This requirement of orthogonality is not trivially fulfilled because in order to be relevant for MD simulations, the DF in a molecule that are not explicitly being scanned should be allowed to relax during both the QM and MM scan, opening the door for concerted motions. Prior to this work, it has been known that full 360° scans with a 15° step size on different dihedrals in a molecules are typically sufficiently orthogonal to unambiguously and relatively robustly fit the different dihedral parameters. Exceptions are cases where strong electrostatic interactions that are partially governed by a different dihedral (e.g. hydrogen bonds associated with an -OH rotation) are established and broken or steric clashes occur during a scan. For detailed work such as the parametrization of biomolecular force fields, it is occasionally even advantageous to perform 2D scans on adjacent dihedral. Yet, as a general rule, relaxed 1D scans work sufficiently well for a majority of dihedrals, especially for the purpose of parametrizing small organic molecules.[4] The same cannot be said of bonds and angles. To parametrize the latter, we propose the use of "3-point scans", as 3 scan points is the minimum necessary to approximate a second derivative or spring constant. In our proposal, one of the scan points is the minimum energy conformation and for the two others, the DF of interest is respectively incremented and decremented by a constant. The problem with scanning bonds and angles in this fashion is the high correlation between the motions of 2 or more DF in relaxed scans (i.e. one DF adjusting in a coherent fashion while a second one is being scanned and *vice versa*), making it impossible for any fitting algorithm to produce meaningful independent values. This issue was so pervasive that it was decided to uniquely perform constrained QM (3-point) scans on bonds and angles, where only the DF being scanned is allowed to vary. However, this poses another problem; one could propose to constrain the corresponding "inactive" DF in the MM scans at their MM values, but those change as the parameters are being optimized, requiring iterative fitting until self-consistency is reached, a situation the present work is seeking to avoid. Instead, said inactive DF were constrained at their *QM* values, under the hypothesis that the MM geometry *after fitting* will be close to the QM geometry.[4] While doing so conveniently reduces the MM scan to the calculation of single-point MM energies on the QM geometries, when fitting dihedrals and rigid DF simultaneously, it introduces an energetic gap between the relaxed sans and the constrained scans because the inactive DF are at their MM values in the former and at their QM values in the latter. In other words, one could say the relaxed scan points are relative to the energy of the MM minimum, while the constrained points are relative to the MM energy of the QM minimum. This was solved by using the group fitting discussed in section 2.11 to independently align points that result from constrained and relaxed scans.

It should be noted that Burger *et al.* recently showed that the orthogonality problem discussed in the present paragraph can be defeated conveniently by MC sampling of molecular conformations.[5] However, this was only demonstrated in 2-dimensional parameter spaces. In the formalism from section 1.1, the rows of $\mathbf{A}$ represent equations, and analogous to the columns of $\mathbf{A}$ (i.e. the response vectors), the set of m row vectors must at least contain a subset of n vectors for which all the pairwise dot products are larger than a small predetermined value in order for the system to not be ill-conditioned. When the m row vectors are chosen randomly (which is essentially what is done in MC conformational sampling), for any given n, one can calculate the number of rows m necessary to have a high predetermined probability P that the system is not ill-conditioned. As it can easily be shown that the required number of samples m grows faster than the dimensionality n, MC is not a solution when performing concerted fitting of a large number of parameters. That said, a lot of practical parameter fitting problems involve small model compounds and modest numbers of parameters. In these cases, MC sampling may potentially be useful to bypass the need for potential energy scans along carefully chosen sets of coordinates.

Independently of the orthogonality question, the width of the scan range for 3-point scans should be chosen carefully; if it is too small, the corresponding energy differences will also be small, causing the LLS procedure to sacrifice the corresponding data points in favor of "more important" ones. This can be counteracted by giving said data points a higher weight w_j (see section 2.11), at the cost of making the fitting procedure somewhat more cumbersome. Small values also have a smaller numerical precision, which cannot be counteracted with weight factors. Conversely, large values will increasingly exhibit the inherent anharmonicity and asymmetry of the QM potential; since the Class I Potential Energy Function cannot explicitly capture these effects, this incurs a risk of overfitting. While these effects were modest in some of our case studies, in other cases, steric clashes and other nonbonded interactions that occur during the scan exacerbated the problem to the point of producing nonsensical results such as negative force constants. Assuming the protocol outlined in the previous paragraph is followed (and ignoring data points greater than 12 kcal/mol above the global minimum in the relaxed dihedral scans[6]), it was empirically found that the best results were obtained by choosing the step size for the bond and angle 3-point scans such that the outer points are between 1 and 3 kcal/mol above the middle (minimum energy) point. This typically corresponded to step sizes of ∼0.05Å and ∼5° for bonds and angles, respectively.

Finally, it should be emphasized that, as discussed in section 2.10, nonzero targets often significantly improve fitted bond and angle parameters. Indeed, since restraining these types of parameters towards zero is questionable, the program developed as part of the present work will set $\sigma_i = 0$ for bonds and angles for which no initial guess is given.

S3 Case studies

S3.1 Analysis of degrees of freedom and symmetry in tetrahydrofuran

Ignoring all substituent atoms including hydrogen, a 5-membered ring has $3n - 6 = 9$ geometrical degrees of freedom. The ring closure makes it impossible to express these degrees of freedom in terms of isolated bonds, angles and dihedral angles; any change in ring geometry can only be expressed as a combination of these redundant internal coordinates. Also, when constraining 2 dihedrals and allowing the ring to relax in the field of the bond and angle potentials only, the remaining 3 dihedrals are rigidly determined, so it can be said that a 5-membered ring has 2 torsional degrees of freedom (more generally $n - 3 - (\#$ in-ring double bonds$)$). These two degrees of freedom can be represented in different ways. For the purpose of vibrational analysis, it is convenient to use a twisting motion that involves 5 dihedrals and an envelope pucker that involves 4 dihedrals, respectively labeled "torsion" and "torsion′" in reference[7]. Another common representation is the pseudorotation angle and puckering amplitude; PES as a function of the former illustrate well how subtle the energy profile of saturated 5-membered rings is.[8] However, for the purpose of fitting dihedral parameters, the objective is to cover the ring's torsional space regardless of whether the order in which different conformations are visited has any physical meaning. For this purpose, it suffices to perform a 2D PES as a function of any two in-ring dihedrals. A similar pragmatic philosophy is adapted for the bonds and angles, as discussed in more detail in the next paragraph. Since the present case study focuses on the 5-membered ring, none of the parameters involving hydrogen atoms are fit. Just as the nonbonded parameters, they are kept at their respective CGenFF values, which were copied from the work in reference[9] during the initial population of the force field.[4]

Because of symmetry, a full bonded description of THF in a Class I force field with the same atom type for all the carbon atoms requires 2 in-ring bond parameters, 3 in-ring angle parameters and 3 in-ring dihedral parameters. Accordingly, the set of target data must cover at least a 8-dimensional conformational space to prevent the fitting problem from being *inherently* ill-conditioned.* This condition can be satisfied by scanning an appropriate subset of 8 out of the ring's 9 degrees of freedom; in this sense, this is an atypical example because a majority of molecules have more bonded parameters than geometric degrees of freedom. On the other hand, most actual parameter optimization projects are extensions of existing force fields and in this context, most new model compounds contain significant numbers of transferred parameters the optimization of which would negatively affect the force field's consistency and transferability. A more common source of ill-conditioned behavior in these cases is the usage of a non-optimal conformational ensemble. Indeed, generating a conformational ensemble that optimally samples the internal coordinates associated with the required parameters without introducing correlation is not straightforward, especially on cyclic systems. At any rate, doing so in the context of the present case study would make the results trivial; instead, we choose to mimic a more realistic use case by using a conformational ensemble that is more aimed at being straightforward to generate than at avoiding correlation.

Another consequence of THF's high degree of symmetry is that for the 2D C-C-C-O dihedral scan, it would in principle suffice to scan only one of the quadrants delineated by the diagonals of figure 4a; the other quadrants could be reconstructed using symmetry operations. However, it is technically more convenient to scan a rectangular domain, in this case half of the figure (i.e. one of the dihedrals was scanned from -48° to +48° and the other one from -48° to 0°, both in steps of 8°). Moreover, if any substituents with rotatable bonds were connected to the ring, doing so would have the added advantage that the resulting redundancy could function as a consistency check. Indeed, while the conformation of a 5-membered ring is fully defined by two dihedrals, in a more general case, relaxed PES are prone to hysteresis in the energy associated with dihedrals that are being relaxed, and repeating a scan in a different direction is a common method to identify and correct such hysteresis. As in the two dihedral scans, the angle scans (figures 4b and 4c) are symmetric around the first bisector of the plot; however, they do not posses the point symmetry (more precisely central symmetry) that is unique to dihedrals in achiral molecules. Again, scanning a triangle is technically inconvenient, so the entire surface was scanned.

References

1. C. I. Bayly, P. Cieplak, W. D. Cornell, and P. A. Kollman, J. Phys. Chem. **97**, 10269 (1993).

---

*Although oft-repeated in the literature, *very strictly spoken*, the system is not necessarily *formally* underdetermined if this condition is not satisfied (as long as the number of scan points is larger than or equal to the number of parameters) because the functional forms of the bond, angle and dihedral terms are different. However, in practice, for reasonably small deviations around the equilibrium, they are good enough approximations of one another to make the system extremely ill-conditioned, so that its behavior is not easily distinguished from underdetermined.

2. O. Guvench and A. D. MacKerell, Jr., J. Mol. Model. **14**, 667 (2008).
3. R. B. Best, X. Zhu, J. Shim, P. E. M. Lopes, J. Mittal, M. Feig, and A. D. MacKerell, Jr., J. Chem. Theory Comput. **8**, 3257 (2012).
4. K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, et al., J. Comput. Chem. **31**, 671 (2010).
5. S. K. Burger, P. W. Ayers, and J. Schofield, J. Comput. Chem. **35**, 1438 (2014).
6. O. Guvench, E. R. Hatcher, R. M. Venable, R. W. Pastor, and A. D. MacKerell, Jr., J. Chem. Theory Comput. **5**, 2353 (2009).
7. P. Pulay, G. Fogarasi, F. Pang, and J. Boggs, J. Am. Chem. Soc. **101**, 2550 (1979).
8. V. M. Rayón and J. A. Sordo, J. Chem. Phys. **122**, 204303 (2005).
9. I. Vorobyov, V. Anisimov, S. Greene, R. Venable, A. Moser, R. Pastor, and A. MacKerell, Jr., J. Chem. Theory Comput. **3**, 1120 (2007).

Table S1: Final fitted parameters for Hexopyranose monosaccharides

```
* Parameters generated by analogy by
* ParamChem least-squares fitting version 0.8.8.0 alpha
* RMSE = 0.561613
*

DIHEDRALS
OD306A   CD31HA   OD31F    HDP1A        1.0146  1     0.00 ! RMSE = 0.561613
OD306A   CD31HA   OD31F    HDP1A        1.0785  2     0.00 ! RMSE = 0.561613
OD306A   CD31HA   OD31F    HDP1A        0.0169  3   180.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD31F    HDP1A        0.3991  1     0.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD31F    HDP1A        0.1491  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD31F    HDP1A        0.5645  3     0.00 ! RMSE = 0.561613
CD31HA   CD31HB   OD31F    HDP1A        0.0231  1   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   OD31F    HDP1A        0.4967  2     0.00 ! RMSE = 0.561613
CD31HA   CD31HB   OD31F    HDP1A        0.2024  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   OD31F    HDP1A        0.3113  1     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   OD31F    HDP1A        0.3404  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   OD31F    HDP1A        0.3597  3     0.00 ! RMSE = 0.561613
CD31HC   CD31HB   OD31F    HDP1A        0.2674  1     0.00 ! RMSE = 0.561613
CD31HC   CD31HB   OD31F    HDP1A        0.4684  2     0.00 ! RMSE = 0.561613
CD31HC   CD31HB   OD31F    HDP1A        0.2486  3     0.00 ! RMSE = 0.561613
CD31HC   CD32A    OD31A    HDP1A        0.7172  1     0.00 ! RMSE = 0.561613
CD31HC   CD32A    OD31A    HDP1A        0.7544  2     0.00 ! RMSE = 0.561613
CD31HC   CD32A    OD31A    HDP1A        0.1744  3     0.00 ! RMSE = 0.561613
OD306A   CD31HC   CD32A    OD31A        1.0465  1   180.00 ! RMSE = 0.561613
OD306A   CD31HC   CD32A    OD31A        0.4194  2     0.00 ! RMSE = 0.561613
OD306A   CD31HC   CD32A    OD31A        0.2015  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HC   CD32A    OD31A        0.1296  1   180.00 ! RMSE = 0.561613
CD31HB   CD31HC   CD32A    OD31A        0.0201  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HC   CD32A    OD31A        0.4119  3   180.00 ! RMSE = 0.561613
CD32A    CD31HC   OD306A   CD31HA       0.0766  1   180.00 ! RMSE = 0.561613
CD32A    CD31HC   OD306A   CD31HA       0.0352  2   180.00 ! RMSE = 0.561613
CD32A    CD31HC   OD306A   CD31HA       0.3231  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   CD32A        0.4627  1   180.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   CD32A        0.1419  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   CD32A        0.0145  3   180.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   CD32A        2.5093  1     0.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   CD32A        0.6924  2   180.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   CD32A        0.1247  3   180.00 ! RMSE = 0.561613
```

```
CD31HC   CD31HB   CD31HB   OD31F        0.1361  1     0.00 ! RMSE = 0.561613
CD31HC   CD31HB   CD31HB   OD31F        0.0384  2   180.00 ! RMSE = 0.561613
CD31HC   CD31HB   CD31HB   OD31F        0.1620  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   CD31HC       0.0732  1     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   CD31HC       0.0551  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   CD31HC       0.0797  3   180.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   CD31HC       0.4129  4   180.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD306A   CD31HC       0.1475  1   180.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD306A   CD31HC       0.0866  2   180.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD306A   CD31HC       0.1668  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HA   OD306A   CD31HC       0.0928  4     0.00 ! RMSE = 0.561613
OD31F    CD31HA   OD306A   CD31HC       0.8500  1   180.00 ! RMSE = 0.561613
OD31F    CD31HA   OD306A   CD31HC       0.0066  2     0.00 ! RMSE = 0.561613
OD31F    CD31HA   OD306A   CD31HC       0.2383  3   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   CD31HB       0.2372  1   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   CD31HB       0.1534  2   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   CD31HB       0.0216  3   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   CD31HB       0.2722  4   180.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   OD31F        0.1236  1     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   OD31F        0.1346  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HB   OD31F        0.1225  3   180.00 ! RMSE = 0.561613
CD31HB   CD31HC   OD306A   CD31HA       0.3593  1   180.00 ! RMSE = 0.561613
CD31HB   CD31HC   OD306A   CD31HA       0.1939  2   180.00 ! RMSE = 0.561613
CD31HB   CD31HC   OD306A   CD31HA       0.3261  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HC   OD306A   CD31HA       0.2151  4     0.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   OD306A       0.0586  1   180.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   OD306A       0.2710  2     0.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HC   OD306A       0.3295  3   180.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HB   OD31F        0.8264  1   180.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HB   OD31F        0.0281  2     0.00 ! RMSE = 0.561613
OD31F    CD31HB   CD31HB   OD31F        0.0961  3     0.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   OD31F        0.1790  1     0.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   OD31F        0.0062  2   180.00 ! RMSE = 0.561613
CD31HA   CD31HB   CD31HB   OD31F        0.8373  3   180.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   OD306A       0.1392  1     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   OD306A       0.0300  2     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   OD306A       0.0307  3     0.00 ! RMSE = 0.561613
CD31HB   CD31HB   CD31HC   OD306A       0.7560  4     0.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   CD31HB       0.1017  1   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   CD31HB       0.0862  2   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   CD31HB       0.0564  3   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   CD31HB       0.1435  4   180.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   CD31HB       0.1531  1   180.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   CD31HB       0.4706  2   180.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   CD31HB       0.4317  3   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   OD31F        0.1350  1   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   OD31F        0.0952  2   180.00 ! RMSE = 0.561613
OD306A   CD31HA   CD31HB   OD31F        0.1673  3     0.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   OD31F        0.5995  1   180.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   OD31F        0.3742  2     0.00 ! RMSE = 0.561613
OD31F    CD31HA   CD31HB   OD31F        0.0036  3   180.00 ! RMSE = 0.561613

END
```