Manual for Determining Periodic Drift Time Using Python Scripts

The Python scripts were developed by Dr. Pattipong Wisanpitayakorn at Mahidol University, Thailand, to assist cyclic ion mobility users in determining periodic drift time (t_p) as outlined in our manuscript [link to be provided upon acceptance].

Step-by-Step Instructions for Using the Script:

1. **Cyclic Ion Mobility Measurements:**
   Perform multiple direct-infusion measurements with stepwise separation times as described in our manuscript.
2. **Processing Raw Files:**
   After acquiring the raw data files from these measurements, process them simultaneously using HDI software.
3. **Locating Output Files:**
   The resulting text files containing peak information will be generated within the imaging folder of the raw files.
4. **Organizing Files:**
   Collect all HDI output files from the measurements into a single folder. We recommend creating a simple command prompt script to automate this process.
5. **File Conversion:**
   The output files from HDI may not have extensions. Use the script `Addtextfile Extension.py` to convert these files into text files.
6. **Location of the scripts:**
   If using the provided sample data, begin from this step. Place the `Calculating_PeriodicDriftTime.py` script and the Excel file containing your target peaks (e.g., `Top_peaks_Human22CwithStandards.xlsx` for our sample data) in the same folder as the text files.
7. **Script Configuration:**
   Several parameters need to be specified in the script:
   a. **ppm_error:** Defines the ppm error margin for matching peaks in the raw data with those in the target Excel file.
   b. **acq_time:** Specifies the acquisition time of the cyclic ion mobility in milliseconds. This value is used for converting arrival time from bin units to milliseconds.
   c. **delta (important):** The script sorts arrival times for each compound from low to high and iteratively determines the pass number. If the difference between consecutive arrival times exceeds the delta value, the pass number increases. Set delta slightly (about 1-2 ms) below the smallest tp expected for your compounds. For lipids and metabolites, a value of 4 ms is generally recommended.
8. **Running the Script:**
   a. **File Conversion:** Converts the text files into Excel files, storing them in the `export_peaks_to_excel` folder.
   b. **Arrival Time Conversion:** Converts arrival times from bin units to milliseconds, saving the results in `export_peaks_to_excel/Converted_DriftTime`.

c. **Peak Matching:** Matches peaks in the raw data with those listed in `Top_peaks_Human22CwithStandards.xlsx`. The script combines data from all files into a single Excel file, `all_arrival_times.xlsx`, which includes m/z values and corresponding arrival times for each separation time. Note: In cases of isobaric compounds (multiple features within 10 ppm of the target m/z), the script records only the arrival times of the feature with the highest signal intensity. For isomeric and isobaric compounds, further script modification may be required.
d. **Arrival Time Analysis:** Determines the arrival time for each pass number from the various separation times and saves the results in `ArrivalTime_by_Pass.xlsx`.
e. **Determining tp:** Performs linear regression on arrival times vs. pass number to calculate t_p and saves the results in `periodic_dt.xlsx`.
f. **Zero-Pass Time (t_0):** For the most accurate results, it is recommended to use the data measured with a separation time of 0.01 ms for the zero-pass time (t_0) (do not use the y-intercept).