# Determining Chromatographic Hydrophobicity Index using Liquid Chromatography – Time of Flight Mass Spectrometry

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## Introduction

In early phase drug discovery, it is essential to determine the physicochemical properties of candidate molecules. Lipophilicity is one such property and is usually determined using octanol: water partition coefficient, to produce a LogD value. Partition coefficient determines a compounds affinity for the lipophilic solvent, relative to the aqueous buffer and it is equal to the ratio of the equilibrium concentration of the two immiscible solvents.

Lipophilicity can also be expressed as the Chromatographic Hydrophobicity Index (CHI). CHI is a value determined by the retention in reverse phase high performance liquid chromatography (RP-HPLC). Historically this has been coupled to an ultra-violet (UV) detector. The retention in reverse phase chromatography is governed by lipophilicity or more precisely hydrophobicity[A]. Reverse phase chromatography is based on a compound's relative affinities for a non-polar stationary phase and a polar mobile phase. The polarity of the mobile phase is altered over time by increasing the proportion of organic solvent present from 0-100%. Using the RP-HPLC method has multiple advantages over the octanol: water partition. These advantages include[B]:

- Less compound is required
- Impurities do not alter the results as they are separated during the run
- Saves time

The CHI value is an approximate % of organic eluent when the compound elutes. This is calculated using a set of calibration compounds with known CHI values. The calibration curve in Figure 1 is used to calculate the CHI values.

The CHI scale of lipophilicity was introduced to convert to a CHI LogD value, using Equation 1[B]:

$$CHI\ LogD = 0.054\ x\ CHI - 1.467$$

Equation 1: the Retention Time of a 10 point Calibration curve against Literature CHI values[C].

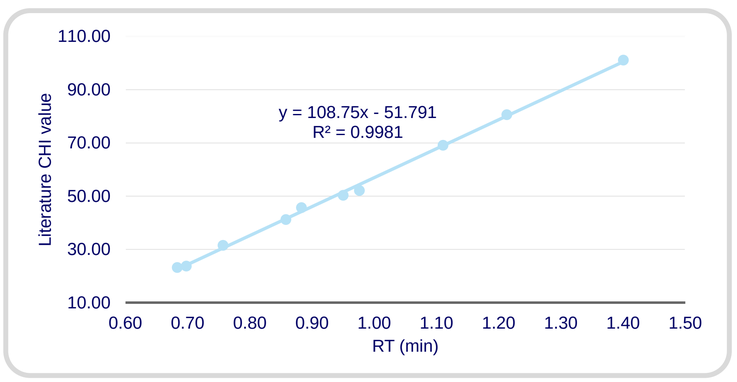


Figure 1: the average Retention Time for 221 injections of a 10 point Calibration curve against Literature CHI values[C].

## Time of Flight Mass Spectrometry

Time of Flight mass spectrometry is a high resolution technique. High resolution mass spectrometry is defined by the Royal Society of Chemistry as "any type of mass spectrometry where the 'exact' mass of the molecular ions in the sample is determined as opposed to the 'nominal' mass (the number of protons and neutrons)"[D].

The use of high-resolution accurate mass spectrometry as a detection method for LC analysis has many advantages over UV detection:

- Sensitivity (500 µM by UV[C] vs. 50 nM by TOF MS)
- Selectivity (m/z measurements accurate to sub 10ppm error) giving confidence in analyte identity for calibration compounds
- Detection of a wide range of chemical species
  - No requirement for a chromophore to be present
- Compound specific method development is not required
- Allows for cassetting

## Analytical Methods

Analysis was conducted using an Agilent 1290 Infinity II LC system with High Speed Pump and Multicolumn Thermostat (Agilent Technologies, Cheshire, UK), PAL RSI autosampler (CTC Analytics, Zwingen, Switzerland), and an ABSciex 6600+ TTOF (ABSciex Ltd, Warrington, UK). The analysis was performed using the following solvent system and gradient:

**Column:** Gemini® 3 µm NX-C18 110Å 50 x 2 mm (Phenomenex, Cheshire, UK)

**Column Temperature:** 40 °C

**Injection Volume:** 4 µL

**Flow Rate:** 800 µLmin$^{-1}$

**Mobile Phase A:** 10 mM ammonium acetate in water (pH adjusted to 7.4 using ammonium hydroxide). Components supplied by Fisher.

**Mobile Phase B:** Acetonitrile supplied by Fisher.

| Time (minutes) | %A | %B |
|---|---|---|
| 0.00 | 100 | 0 |
| 0.10 | 100 | 0 |
| 1.40 | 0 | 100 |
| 1.80 | 0 | 100 |
| 1.85 | 100 | 0 |
| 2.40 | 100 | 0 |

### MS Parameters

| | |
|---|---|
| Scan Type | TOF MS |
| Polarity | Positive |
| Ionisation Mode | TurboIonSpray |
| Scan Range (m/z) | 50-1000 |
| Accumulation time | 50 ms |

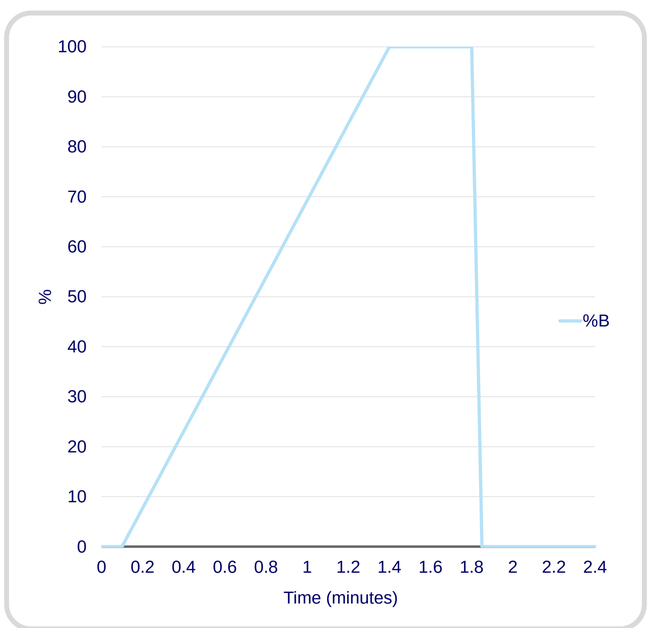


Figure 2: LC gradient profile

## Sample Preparation

All compounds were supplied by either Sigma Aldrich or Alfa Aesar. Each compound was dissolved individually in DMSO to a concentration of 1 mM. The 1 mM samples were placed into individual wells, in a 384-well PP 2.0 microplate Echo qualified (Labcyte Inc, California, USA). Using the Echo 665T (Labcyte Inc, California, USA) to dispense low volumes up to 2.5 nL of sample into a shallow well 96-well plate. The Echo is used due to the fast, accurate, and precise transfer of compounds, which reduces the chances of manual error. This method of analysis is ideal for compounds with limited stocks.

## Data Processing

An in-house processing software has been developed to take the raw data produced from the TTOF and generate chromatograms. This processing tool is able to:

- Integrate chromatograms
- Highlight any irregularities
- Create a 10 point calibration curve (Figure 1)
- Generate individual CHI values for test compounds dependent upon the RT of the compound elutes at against the calibration curve
- CHI LogD (Equation 1)

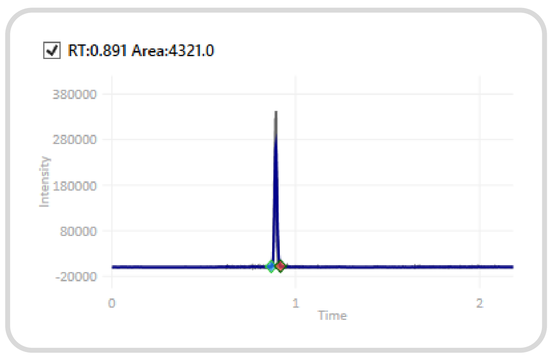


Figure 3: An example chromatogram produced by the in-house processing software.

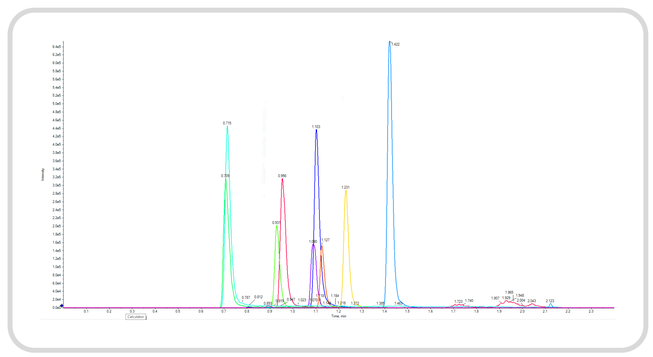

Figure 4: Shows the TTOF Total ion chromatogram of the calibration compounds.

## Results

The calibration compounds were injected 221 times across 2 different instruments to determine the robustness of the method for instrument transfer. The average retention times for the calibration compounds were plotted against their literature CHI values[C] (Figure 1) and produced a calibration curve with an $R^2$ value of 0.9981. Proving there is good correlation between the literature[C] and our processing method. We also calculated CHI value for each of the calibration compounds using the gradient and then calculated a %RSD of the CHI values (Figure 5). The lowest %RSD is 0.53% and the largest %RSD is 3.63%, this %RSD range shows that the data is of an acceptable level of reproducibility. We then used the calibration compounds to calculate a CHI value of 27 compounds and compare them against their Literature CHI values[C] (Figure 6), the correlation produced a $R^2$ value of 0.9950, proving that our current method of producing CHI values is consistent with the literature[C].

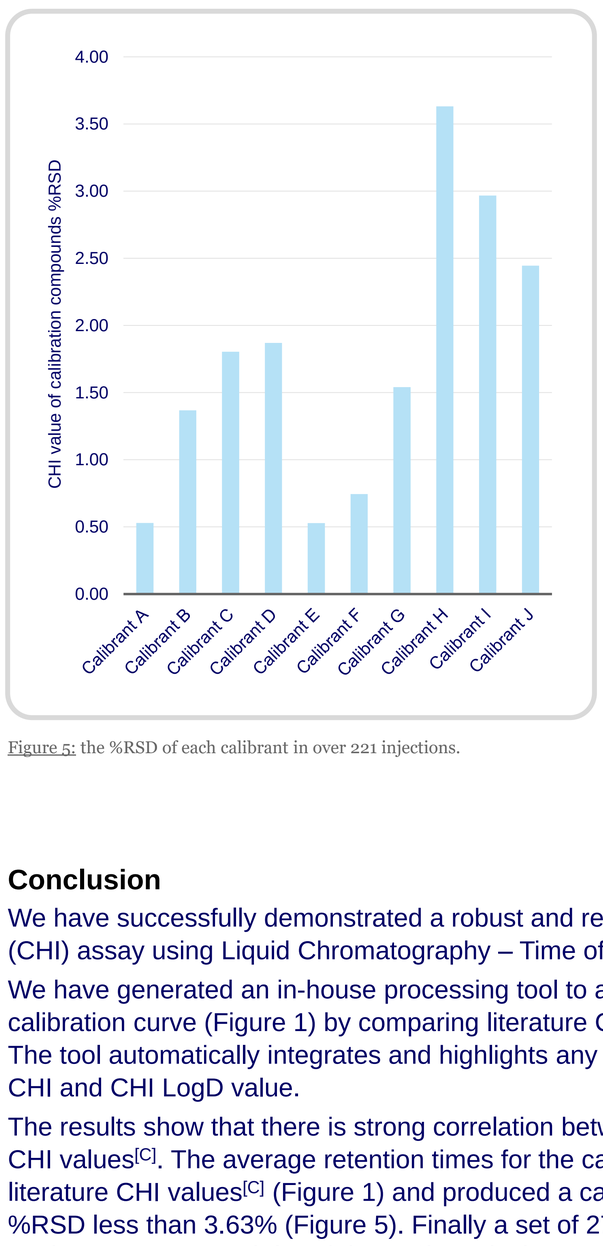


Figure 5: the %RSD of each calibrant in over 221 injections.

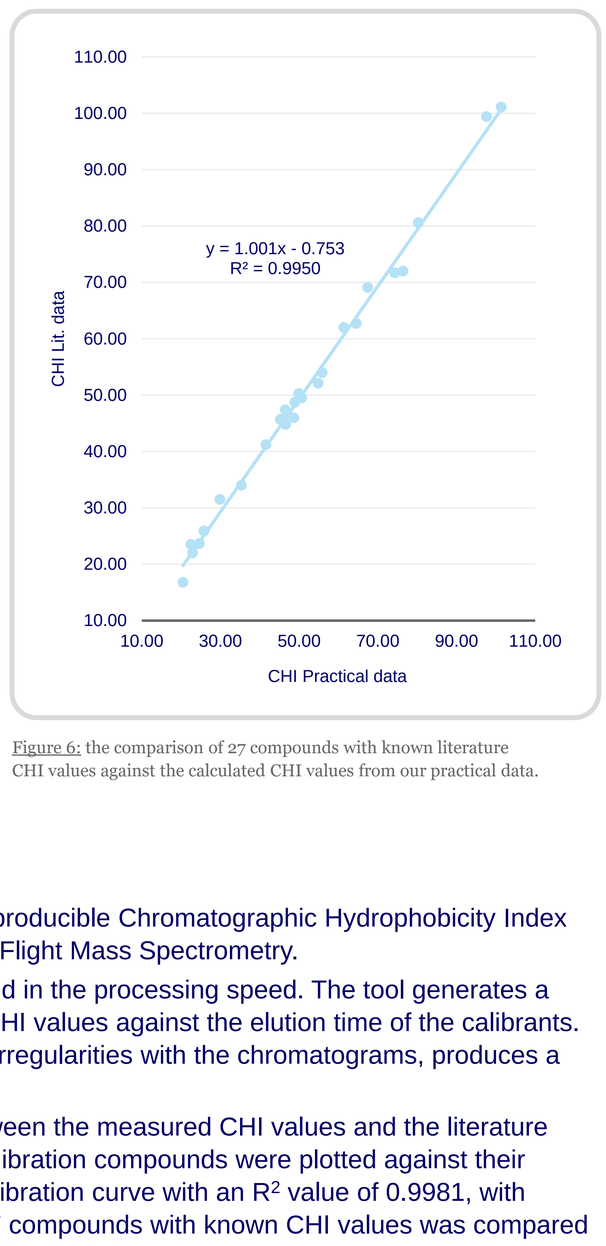


Figure 6: the comparison of 27 compounds with known literature CHI values against the calculated CHI values from our practical data.

## Conclusion

We have successfully demonstrated a robust and reproducible Chromatographic Hydrophobicity Index (CHI) assay using Liquid Chromatography – Time of Flight Mass Spectrometry.

We have generated an in-house processing tool to aid in the processing speed. The tool generates a calibration curve (Figure 1) by comparing literature CHI values against the elution time of the calibrants. The tool automatically integrates and highlights any irregularities with the chromatograms, produces a CHI and CHI LogD value.

The results show that there is strong correlation between the measured CHI values and the literature CHI values[C]. The average retention times for the calibration compounds were plotted against their literature CHI values[C] (Figure 1) and produced a calibration curve with an $R^2$ value of 0.9981, with %RSD less than 3.63% (Figure 5). Finally a set of 27 compounds with known CHI values was compared against our measured data (Figure 6) producing an $R^2 = 0.9950$.

References:

A – A. Vailaya, C. Horváth. Solvophobic theory and normalized free energies of nonpolar substances in reversed phase chromatography. J. Phys. Chem. B 101 (1997) 5875-5888

B – K. Valko, Lipophilicity and biomimetic properties measured by HPLC to support drug discovery, J. Pharm. Biomed. Anal. 130 (2016) 35-54

C – Hollósy, F.; Valkó, K.; Hersey, A.; Nunhuck, S.; Kéri, G.; Bevan, C. Estimation of Volume of Distribution in Humans from High Throughput HPLC-Based Measurements of Human Serum Albumin Binding and Immobilized Artificial Membrane Partitioning *J. Med. Chem.* 2006, 49, 24, 6958-6971

D – Royal Society of Chemistry, High-resolution mass spectrometry, https://www.rsc.org/publishing/journals/prospect/ontology.asp?id=CMO:0000498&MSID=c3tc00801k, Accessed 25th February 2020