

Determination of Log P for Compounds of Different Polarity Using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System

Application Note

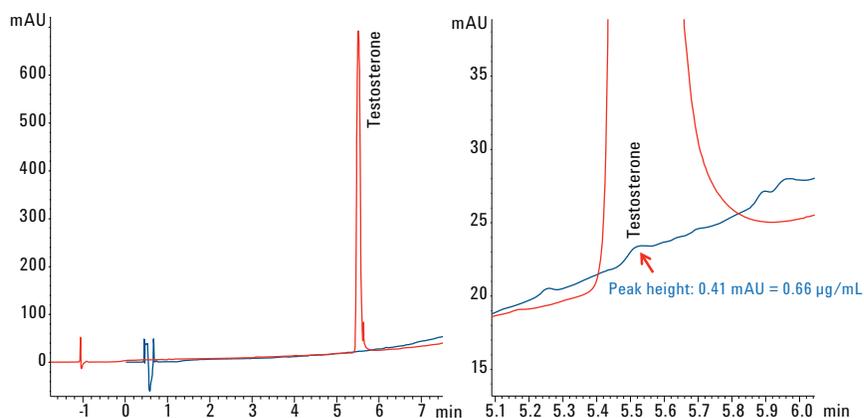
Small Molecule Pharmaceuticals & Generics

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Abstract

The determination of log P, the distribution of a compound between water and octanol, is frequently used in the pharmaceutical industry to evaluate the lipophilicity of a compound. Especially if the distribution between the water and the octanol phase is significantly different, the linear range of the detector must be extremely wide, or the applied injection volumes have to be adjusted. Conversely, high sensitivity is also needed for the evaluation of small peaks. The Agilent 1200 Infinity Series High Dynamic Range Diode Array Detector Impurity Analyzer System covers a wide linear range, typically up to 6,000 mAU. In addition, the 1200 Infinity Series HDR-DAD typically shows a low noise behavior and allows the quantitation of peaks with peak heights below 0.5 mAU. Both enable the quantitation of very high concentrations and very low concentrations using the same injection volume.



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Introduction

The distribution of a drug between water and octanol is one criterion to determine how lipophilic the compound is. The distribution coefficient (log P) gives an indication of how easily the drug is adsorbed, how strong the effect is, how long it remains in the body in an active form, and how it is metabolized and excreted.

The partition coefficient is a ratio of concentrations of unionized compound between the two solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is adjusted such that the predominant form of the compound is unionized. The logarithm of the ratio of the concentrations of the unionized solute in the solvents is called log P. The log P value is also known as a measure of lipophilicity¹. The distribution coefficient for ionizable compounds depends on the pH. The pharmaceutical industry measures the log P value at pH = 7.4. This is the physiological pH of blood serum. The equation typically used is:

$$\log P_{7.4}^{\text{octanol/water}} = \log \left(\frac{\text{concentration}_{\text{octanol}}}{\text{concentration}_{\text{water at pH 7.4}}} \right)$$

The EPA guidelines² give a recommendation on how to perform a log P calculation using HPLC.

One problem using the HPLC approach is that one phase might contain approximately the complete amount of the dissolved drug, whereas the other phase only contains traces. In this case, it is advantageous that the HPLC detection system provide a wide linear range and also high sensitivity. The Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System fulfilled both demands. Typically, the linear range goes up to 6,000 mAU, and peaks below 0.5 mAU are detectable³.

This Application Note shows that the log P values of compounds with different lipophilicity were determined using the 1200 Infinity Series HDR-DAD Impurity Analyzer System.

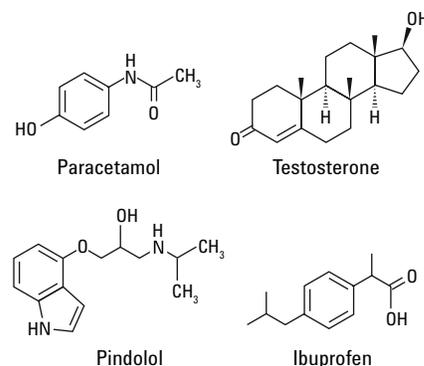
Experimental

The following instruments were used:

- Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System
- Agilent 1290 Infinity DAD with 60-mm cell (G4212A)
- Agilent 1290 Infinity DAD with 3.7-mm cell (G4212A)
- Agilent High Dynamic Range DAD Solution Kit (G2199AA)
- Agilent 1290 Infinity Thermostated Column Compartment (G1316C)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Quaternary Pump (G4204A)

Compounds analyzed

Testosterone (48 mg/10 mL), Pindolol (36.7 mg/10 mL), Ibuprofen (78.5 mg/10 mL), and Paracetamol (79 mg/10 mL), purchased from Sigma Aldrich, Germany



Chromatographic conditions

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 4.6 × 50 mm, 3.5 μm (p/n 959943-902)
Flow rate	1 mL/min
Mobile phases	A) Water B) Acetonitrile C) 1 % TFA
Gradient	at 0 minutes 5 % B, 10 % C, at 8 minutes 90 % B, 10 % C
Run time	10 minutes
Post time	3 minutes
Temperature	30 °C
Detection	230/10 nm Ref=off, 20 Hz
Injection volume	1 μL

Sample preparation

1. The water phase was adjusted to pH 7.4 with phosphate buffer and saturated with octanol.
2. The octanol phase was saturated with water.
3. Both phases were left for separation for 24 hours.
4. Compounds were weighed and dissolved in 10 mL of water at pH 7.4 and octanol = 50/50.
5. The phases were shaken and left for separation for 24 hours.
6. A solution of 1 mL of the water and 1 mL of the octanol phase was transferred into a 1.5 mL vial and analyzed.

Calibration concentrations

	Paracetamol (µg/mL)	Ibuprofen (µg/mL)	Pindolol (µg/mL)	Testosterone (µg/mL)
Stock	7,700	7,850	3,670	13,663
Dilution 1	3,850	3,925	1,835	2,732.6
Dilution 2	1,540	1,570	734	546.52
Dilution 3	308	314	146.8	109.304
Dilution 4	61.6	62.8	29.36	21.8608
Dilution 5	12.32	12.56	5.872	4.37216
Dilution 6	2.464	2.512	1.1744	0.874432

Acquisition and evaluation software

Agilent OpenLAB CDS ChemStation
Rev.C.01.05

Results and Discussion

The 1200 Infinity Series HDR-DAD Impurity Analyzer System was configured during instrument configuration. Both detectors were clustered. The delay volume of the capillary connecting both detectors was determined and filled in the configuration table. In the user interface, both detectors appear as one detector (Figure 1).

The following experiments were done to determine log P:

- Calibration tables were generated to determine the linearity range and sensitivity of the method.
- The water and octanol phase were analyzed to determine the concentrations in both phases for testosterone, paracetamol, ibuprofen, and pindolol.
- Log P was calculated.

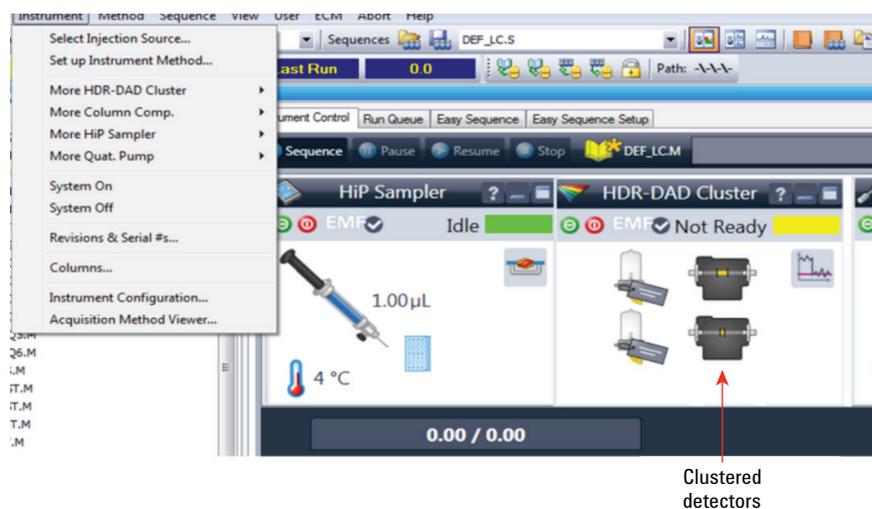
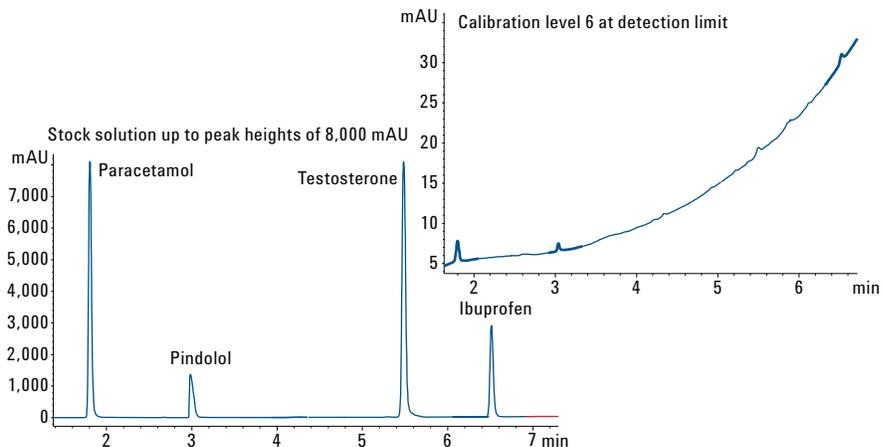


Figure 1. User interface in the Agilent ChemStation.

Linearity and Limit of Detection (LOD)

Linearity was measured using calibration standards from the stock solution down to level 6. The stock solution was the highest concentration, and level 6 was the lowest concentration (Figure 2). The stock solution resulted in peak heights of approximately 8,000 mAU. The lowest calibration level was close to the LOD, with peak heights smaller than 1 mAU.

The 1200 Infinity Series HDR-DAD typically provides a 30-times enhanced linear range compared to conventional DADs³. The calibration curves (Figure 3) provided sufficient linearity for all compounds.



The highest calibration level represents:

Paracetamol = 7,700 ng injected amount
 Pindolol = 3,670 ng injected amount
 Ibuprofen = 7,850 ng injected amount
 Testosterone = 13,663 ng injected amount

The lowest calibration level represents:

Paracetamol = 2.5 ng injected amount
 Pindolol = 1.3 ng injected amount
 Ibuprofen = 2.5 ng injected amount
 Testosterone = 0.874 ng injected amount

Figure 2. Chromatogram of highest and lowest calibration concentration of paracetamol, pindolol, testosterone, and ibuprofen.

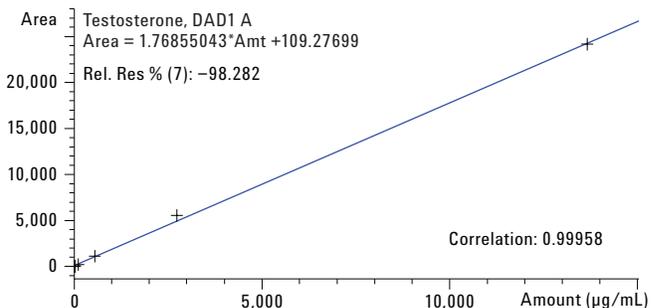
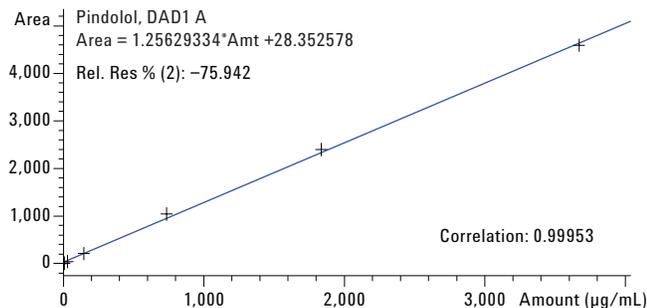
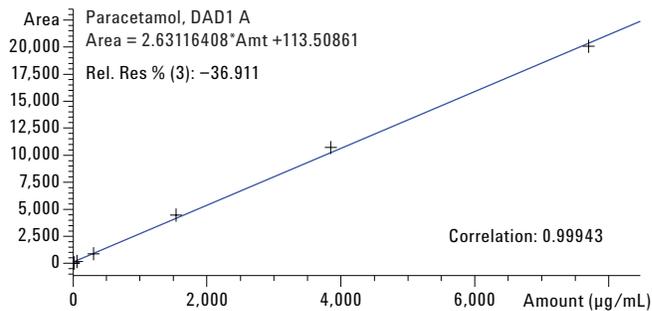
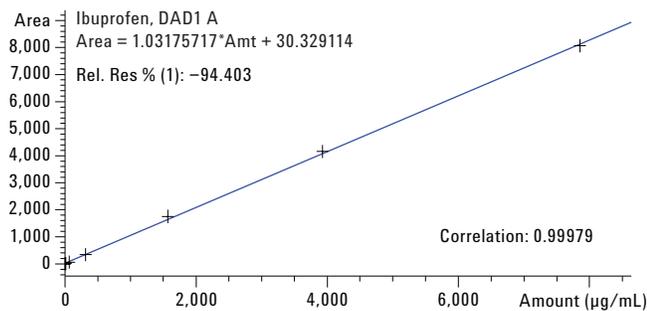


Figure 3. Calibration curves.

Analysis of the water and octanol phase

Testosterone is a compound which is almost nonsoluble in water. Consequently, the peak in the water phase was very low (Figure 4). Whereas the testosterone peak in the octanol phase was approximately 700 mAU high, the peak in the water phase was only 0.41 mAU high.

Paracetamol showed nearly equal distribution between water and octanol. Pindolol dissolved slightly better in water, and ibuprofen dissolved significantly better in octanol (Figure 5). The peak heights for ibuprofen and paracetamol were ~2,500 and ~4,500 mAU, far beyond the linear range of a conventional DAD. Using the 1200 Infinity Series HDR-DAD quantitation was possible with reliable results even for these high concentration ranges.

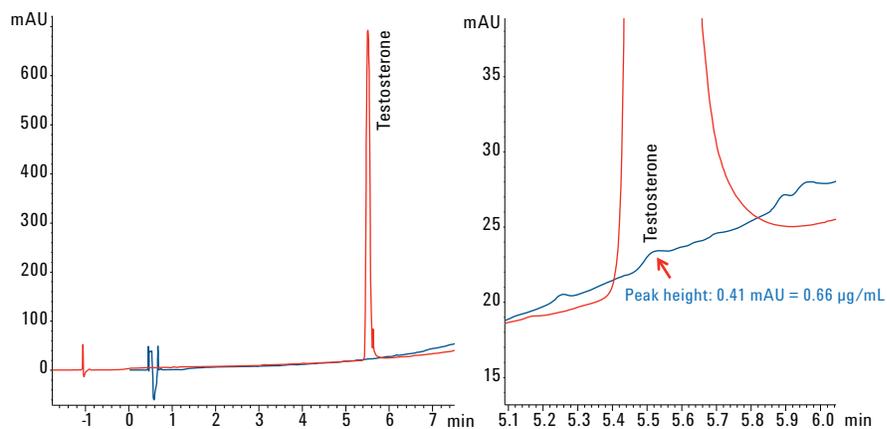


Figure 4. Analysis of testosterone in the octanol and water phase.

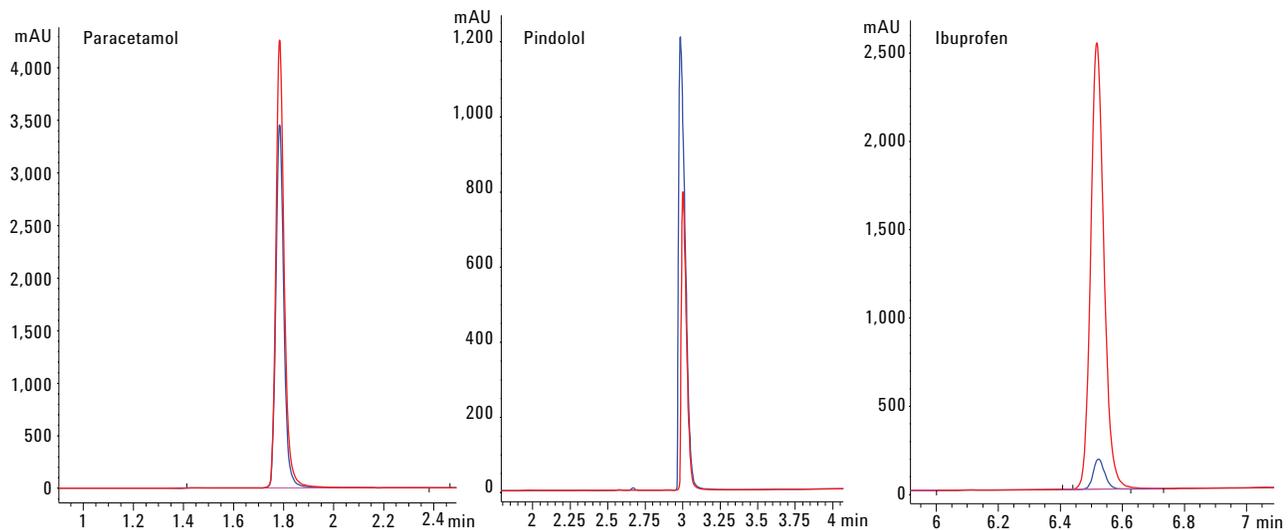


Figure 5. Distribution between water (blue) and octanol (red) for pindolol, ibuprofen, and paracetamol.

Calculation of log P

The log P values were calculated based on the evaluated concentration in the water and octanol phase. Except for paracetamol, the calculated values showed good correlations with data from the literature⁴ (Table 1).

Conclusion

Determination of log P was done using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System. Quantitation of significantly different concentration levels present in the water and the octanol phase were performed without the need of injection volume adaption. Peak heights up to 8,000 mAU were calibrated and quantified due to the wide linear range of the 1200 Infinity Series HDR-DAD. Conversely, peaks as low as 0.41 mAU were quantified due to the low noise behavior of the 1200 Infinity Series HDR-DAD.

Table 1. Calculation of log P.

Compound	Concentration (octanol) (µg/mL)	Concentration (water at pH = 7.4) (µg/mL)	Partition coefficient	Log P at pH = 7.4	Log P at pH = 7.4 from literature
Ibuprofen	7,327.75	391.79	18.703	1.27	1.37
Testosterone	1,259.39	0.66	1,920.098	3.28	3.29
Paracetamol	3,775.25	2,918.93	1.293	0.11	0.51
Pindolol	1,575.73	3,008.82	0.524	-0.28	-0.21

References

1. Wikipedia. Partition Coefficient. http://en.wikipedia.org/wiki/Partition_coefficient (accessed February 10th, 2014).
2. United States Environmental Protection Agency: Prevention, Pesticides and Toxic Substances, (7101), EPA 712-C-96-038, August 1996.
3. Huesgen, A.G., Comparison of sensitivity and linearity of the Agilent 1200 Infinity Series high Dynamic Range Diode Array Detector Solution and the conventional Agilent 1290 Infinity DAD, *Agilent Technologies Application Note*, publication number 5991-1890EN, 2013.
4. Kerns, E.H., *et al.*, Pharmaceutical profiling method for lipophilicity and integrity using liquid chromatography–mass spectrometry, *Journal of Chromatography B*, 2003, 791:381–388.

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