Determination of octonal-water partition coefficients using the Agilent 220 micro plate sampler

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Abstract

The octanol-water partition coefficient (K_{ow}) has been widely accepted as an indication of the distribution of analytes into biological membranes. It has therefore become one of the most commonly reported physical properties of drugs, pesticides and other chemicals. This is not surprising since compounds must be able to traverse or at least partition into biological membranes[1] in order to have biological activity. The determination of K_{ow}, or more specifically log K_{ow}, can be either done by the shake-flask method or directly by reverse phase HPLC. For the second method log K_{ow} can be calculated from the capacity factor k', which depends on several factors such as composition of mobile phase. Therefore compounds with known K_{ow} have to be used as "standards" to determine the partition coefficient of structurally related compounds. Since such standards are not always available and the calculated results are sometimes incorrect, the determination of K_{ow} with the shake-flask method is still common in the pharmaceutical industry.

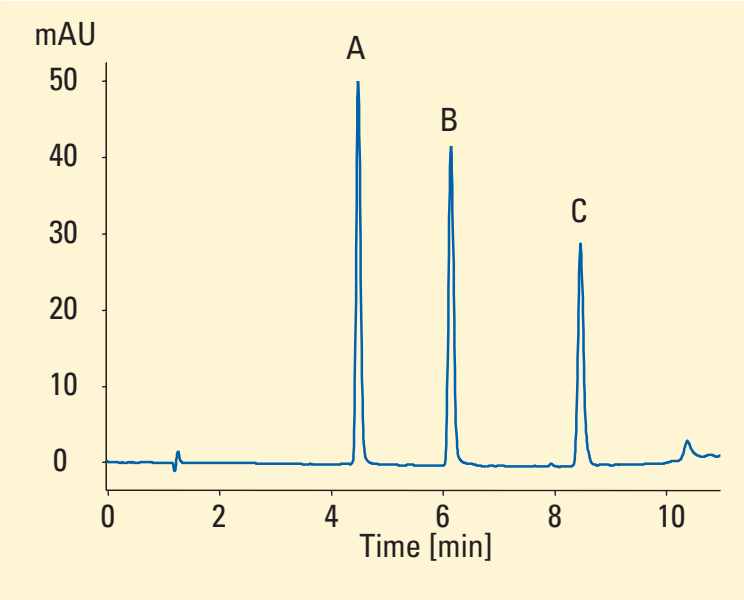

Figure 1
Three structurally related compounds A, B and C

Conditions

Column
4 × 125 mm Hypersil ODS, 5 µm
Mobile phase
A = water, B = acetonitrile
Flow rate
1.0 mL/min
Gradient
at 0 min 20 % B
at 10 min 80 % B
at 11 min 20 % B
UV detector
diode array detector, 254 nm/16
(reference 360 nm/100)
Column compartment temperature
25° C
Stop time
11 min
Post time
5 min
Injection volume
5 µl

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In this application brief the determination of K_{ow} is described for three structurally related compounds by the shake-flask method. The concentration of the compounds in the water and octanol phase is determined by HPLC directly from the shake flasks, which are conventional 2-ml vials. Samples from the octanol and water phase were taken from the same vial using the needle offset feature of the micro plate sampling software. For the quantitative measurements a three point calibration was done for each compound. The calibration table was saved with the method.

For sample preparation about 10 mg compound were dissolved in 10 ml water saturated octanol. 700 µl of the solution were transferred into a conventional 2-ml vial, 700 µl octanol-saturated water were added and the vial was capped. The resulting two-phase mixture was inverted repeatedly in a shaker for about one hour. Then the samples were centrifuged for about 10 minutes to make sure that possible emulsions were removed.

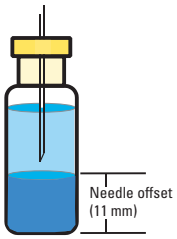

Figure 2
Needle offset

The vials were placed in a rack on the Agilent 220 micro plate sampler and two autosampler methods were set up. The first method was set up to take a sample from the upper octanol phase. Therefore, a needle offset of 11 mm (figure 2) was entered in the *Draw Sample* window of the *Sampler Parameters* screen. A 5-µl sample loop with complete loop fill was used for injection. The second method was set up to analyze a sample from the lower aqueous phase. Therefore, no needle offset was required.

Results

Compound	Conc. (octanol) [mg/L]	Conc. (water) [mg/L]	K_{ow}	Log K_{ow}
A	774.78	23.08	33.56	1.53
B	766.88	8.53	89.86	1.95
C	829.00	6.50	127.59	2.11

Conclusion

The determination of K_{ow} by the shake flask method was shown using the Agilent 220 micro plate sampler and the Agilent 1100 Series LC system. The partition was done in a conventional 2-ml vial from which the compound amounts in the octanol and water phase were directly measured. This was achieved using the needle offset feature of the micro plate sampling software and the Agilent 220 micro plate sampler.

Equipment

Agilent 1100 Series

- Binary pump
- Vacuum degasser
- Thermostatted column compartment
- Diode array detector, standard flow cell
- 220 micro plate sampler
- Agilent ChemStation
- Micro plate sampling software

Column

- Hypersil ODS, 4 x 125 mm, 5 µm (part number 7992618-564)
- Guard cartridges: Hypersil ODS, 4 x 4 mm, 5 µm (part number 7992618-504, 10/pk)

References

1. S. Griffin, S. Grant Wyllie, J. Markham, J. of Chromatography, **1999**, 864, 221-228

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