Quantification of Diclofenac and Ibuprofen by a Vibrio Fischeri Bioluminescence Assay

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3.1 Quantification of Diclofenac and Ibuprofen by a Vibrio Fischeri Bioluminescence Assay

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Abstract
We present a video-densitometric quantification method for the pain killer known as diclofenac and ibuprofen. These non-steroidal anti-inflammatory drugs were separated on cyanopropyl bonded plates using CH2Cl2,methanol,cyclohexane (95+5+40, v/v) as mobile phase. The quantification is based on a bio-effective-linked analysis using vibrio fischeri bacteria. Within 10 minutes a CCD-camera registers the white light of the light-emitting bacteria. Diclofenac and ibuprofen effectively suppress the bacterial light emission which can be used for quantification within a linear range of 10 to 2000 ng. The detection limit for ibuprofen is 20 ng and the limit of quantification 26 ng per zone. Measurements were carried out using a 16-bit ST-1603ME CCD camera with 1.56 megapixels [from Santa Barbara Instrument Group, Inc., Santa Barbara, USA]. The range of linearity covers more than two magnitudes because the extended Kubelka-Munk expression is used for data transformation [1]. The separation method is inexpensive, fast and reliable.

Ibuprofen is named after its chemical description: iso-butyl-propanoic-phenolic acid. Both pain killers are world-wide in use and both substances are stable in aqueous solution. Both substances are mainly excreted in the urine.

Diclofenac and Ibuprofen in the Environment
Ibuprofen is the third most consumed drug in the world. It is well known that wastewater treatment plants only remove 60 to 90 percent of this drug. The concentration of ibuprofen in wastewater exceeds 1 µg per L [2]. The same is true for diclofenac. Both substances can be detected at low levels (1-6 ng per L) even in drinking water [2]. In Germany, diclofenac and ibuprofen have been particularly identified as widespread contaminants of the water cycle [2]. In long-term monitoring investigations of sewage and surface water samples, diclofenac was identified as one of the most important pharmaceutically active compounds present in water. [2 - 4]

Diclofenac is known as a very problematic compound. The use of diclofenac in animals has been reported to have led to a sharp decline in the vulture population in the Indian subcontinent, 95 % decline in 2003, [5] 99.9 % decline in 2008. The loss of tens of millions of vultures over the last decade has had major ecological consequences across the Indian subcontinent, posing a potential threat to human health [5]. The purpose of this work is to present a simple but very sensitive Thin-Layer Chromatography method for the quantification of ibuprofen and diclofenac in aqueous environmental samples.

Exoerunebtak secction
For direct video-densitometric evaluation a ST-1603ME CCD camera with 1.56 megapixels from Santa Barbara Instrument Group, Inc., Santa Barbara, USA was used. The camera was mounted with a Kodak KAF-1603ME CCD pixel array containing 1530 X 1020 pixel. The array size is 13.8 X 9.2 mm with a pixel size of 9 X 9 microns. The camera uses a 16 bit A/D converter and a high speed USB interface. The camera was used in combination with a Schneider SKR KMP Xenoplan 28/2,0 – M30,5 lens. For plate evaluation the CCD-array was cooled to –5°C. After separation and staining the HPTLC-plate is placed below the camera at a distance of 30 cm. This distance is adjusted so that 8.5 cm are detected by 1020 pixel providing a resolution of 83.3 µm per pixel. A single mm separation distance is measured by 12 diodes producing 12 data points. The time of 600 seconds is necessary to measure the full 16 bit range.

Results and discussion
The compounds ibuprofen and diclofenac were separated on cyanopropyl-bonded HPTLC-plate to a distance of 70 mm from the starting point, using CH2Cl2, methanol, cyclohexane (95+5+40, v/v) as mobile phase. All zones were measured by averaging 9 diodes to single densitograms. Paracetamol was measured at 300 nm and the compounds chloramphenicol and diclofenac were measured at 240 nm. Ibuprofen shows nearly no light absorption. Even at an amount of 2000 ng per zone the signal height is so small that noise is clearly visible.

In Figure 3.1-1 the ibuprofen peak at 42.5 mm separation distance is plotted for an amount of 767 ng but looks really tiny. The detection limit (LOD) for ibuprofen is 180 ng per zone, using UV-spectra for evaluation. The limit of quantification (LOQ) for ibuprofen was calculated to 210 ng. The detection limit for diclofenac (the peak at left, beside the ibuprofen signal) was estimated to 33 ng per zone and its LOQ-value is 42 ng per zone. All data were calculated according to the Funk-algorithm [9].

Dipping the plate in a vibrio fischeri solution, wiping the surface to remove all the water and measuring over a period of 10 minutes gave no light suppression of chloramphenicol and paracetamol zones. In contrast to that, luminescences of the vibrio fischeri bacteria were strongly suppressed by ibuprofen and diclofenac. This effect is clearly visible in Figure 3.1-1. The peaks for of diclofenac at 39 mm and ibuprofen at 42.5 mm separation distance show no noise in comparison to the non-dipped zones detected at 224 nm. It can also be seen that ibuprofen suppresses the vibrio fischeri bacteria more effectively than diclofenac. Both zones contain nearly the same amount of pain killer but the ibuprofen peak in Figure 3.1-1 is larger than the diclofenac signal.

Treatment with luminescence bacteria seems to be a specific and very sensitive method to obtain linear calibration plots for the quantification of ibuprofen and diclofenac (see Figure 3.1-2). The limit of detection (LOD) for ibuprofen is 20 ng per zone and for diclofenac 89 ng per zone. The limit of quantification (LOQ) for ibuprofen is 26 ng per zone and for diclofenac 129 ng per zone.

The method is suitable for quantifying both compounds in aqueous samples at a very low level. Due to its poor light absorption ability the quantification of ibuprofen at low level was previously not possible. In terms of LOD and LOQ the method provides a real improvement of ibuprofen quantification by TLC. An additional advantage of the method is its specificity, because nearly all other compounds show no effect on vibrio fischeri bacteria.

$$TMD(k) = k\left(\frac{1}{R} - R\right) + (R-1) = \frac{a}{(1-a)} \quad (1)$$

k: backscattering factor (k≥0 and k≤1)
a: absorption coefficient

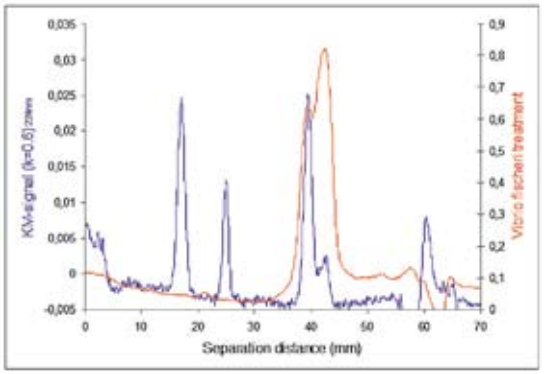

Fig. 3.1-1: *Plotted are the densitograms of UV-evaluation at 224 nm and a vibrio fischeri treatment. The compounds diclofenac and ibuprofen are located at 39 mm separation distance (750.8 ng per zone) and at 42.5 mm separations distance (767 ng per zone)*

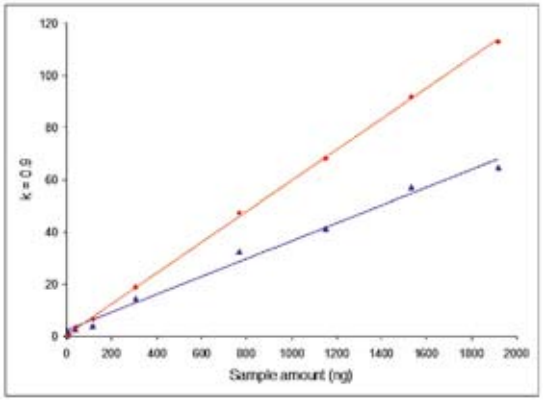

Fig. 3.2-1: *Plotted are the densitograms of UV-evaluation at 224 nm and a vibrio fischeri treatment. The compounds diclofenac and ibuprofen are located at 39 mm separation distance (750.8 ng per zone) and at 42.5 mm separations distance (767 ng per zone)*

Conclusion

The combination of TLC-separation using cyanopropyl-bonded plates and luminescencent bacteria make sensitive quantification of ibuprofen and diclofenac possible. The method can be used for quantification of both substances in aqueous samples.

References

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