

Development of Field-Portable Immunoextraction/HPLC Systems for Herbicides and Other Environmental Contaminants

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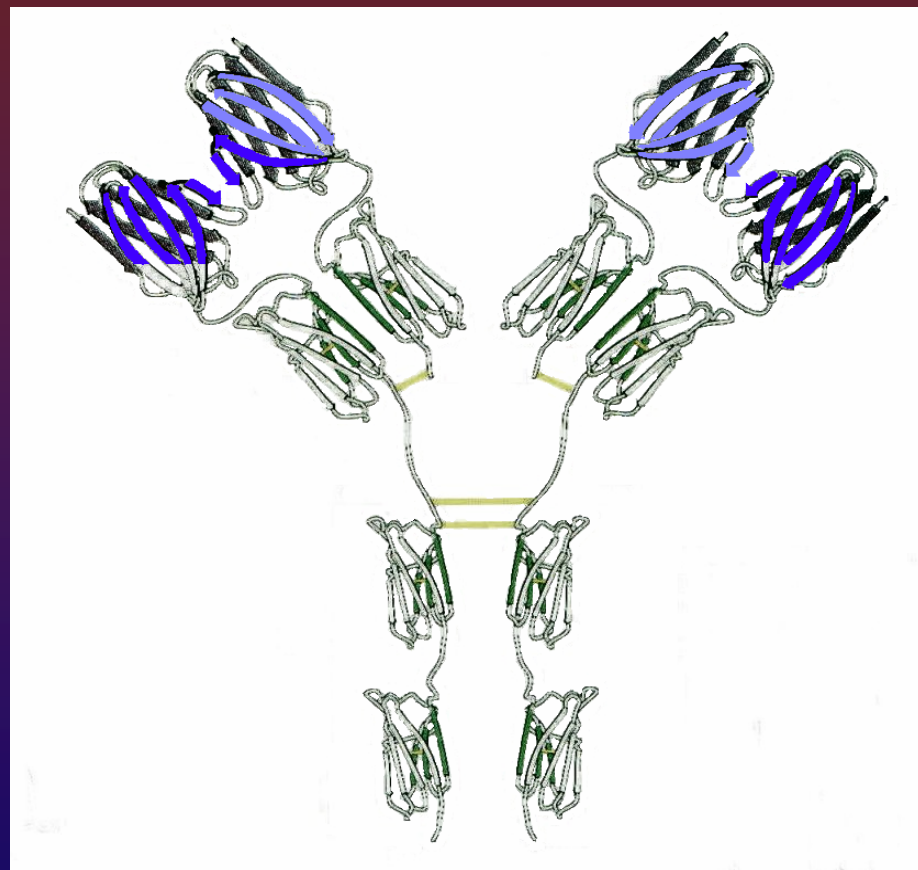
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Immunoassay Detection of Herbicides

Possible Advantages

- Highly selective for a class of compounds
- Can be used with many analytes
- Can be used with many sample types
- Can result in low detection limits

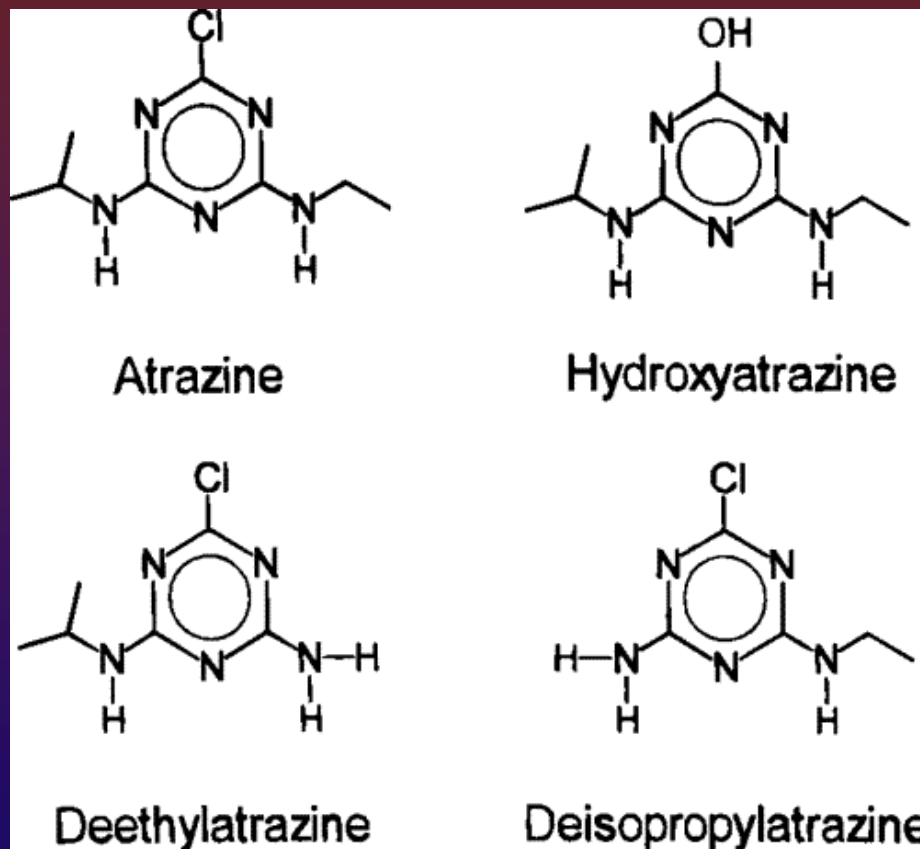


Structure of Immunoglobulin G (IgG)

Immunoassay Detection of Herbicides (Cont'd)

Possible Disadvantages

- Interferences from structurally similar compounds
- Often manual method
- Can suffer from low precision & poor reproducibility



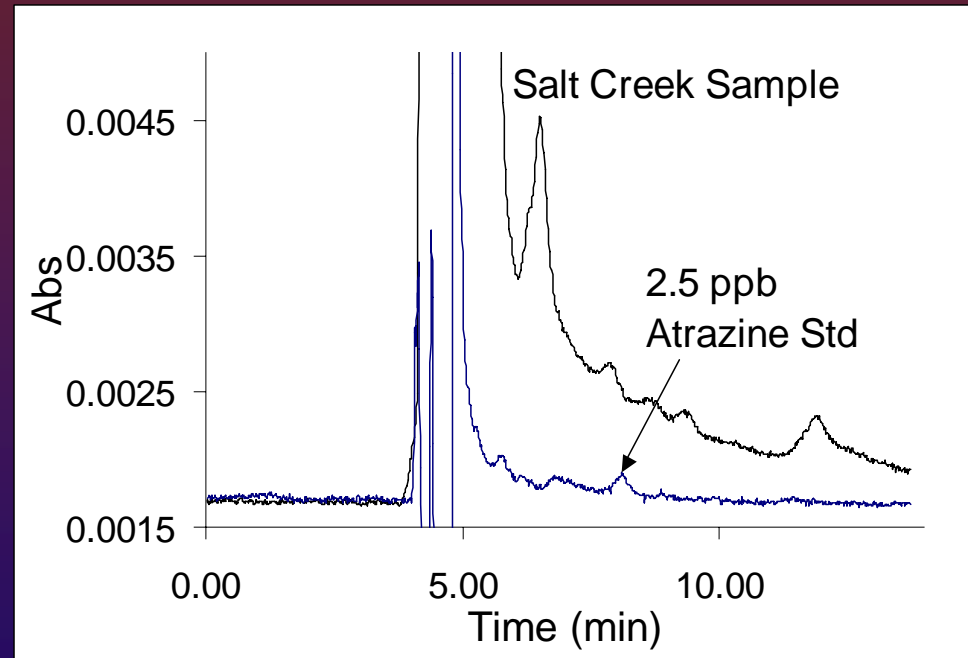
Atrazine and its Major Degradation Products



Immunoextraction/HPLC Detection of Herbicides & Environmental Agents

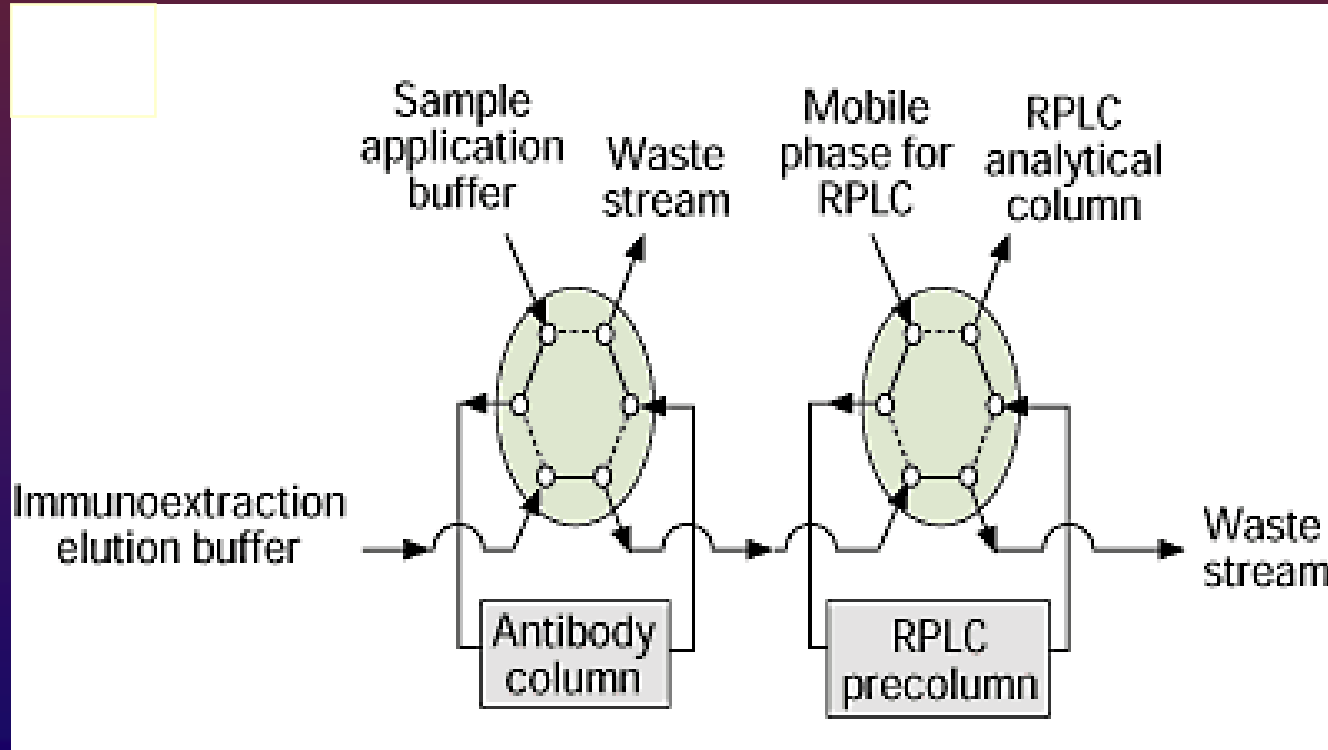
Possible Advantages

- Retains selectivity of immunoassays & their ability to be used with many analytes/samples
- Adds the precision, speed and ability of HPLC to give automated systems

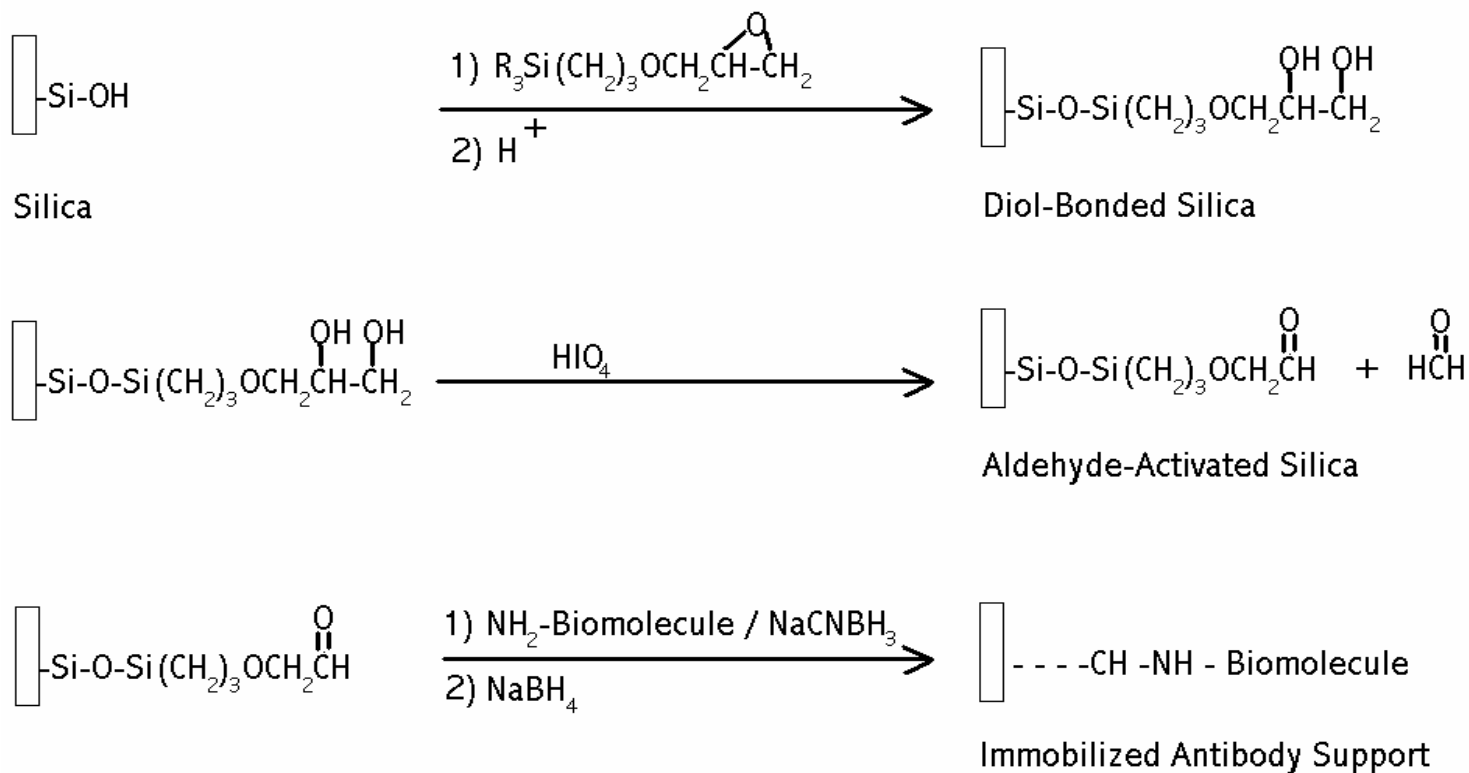


Example of the analysis of atrazine in river water by immunoaffinity/HPLC

Scheme for On-Line Immunoextraction/HPLC (using Reversed-phase Liquid Chromatography)



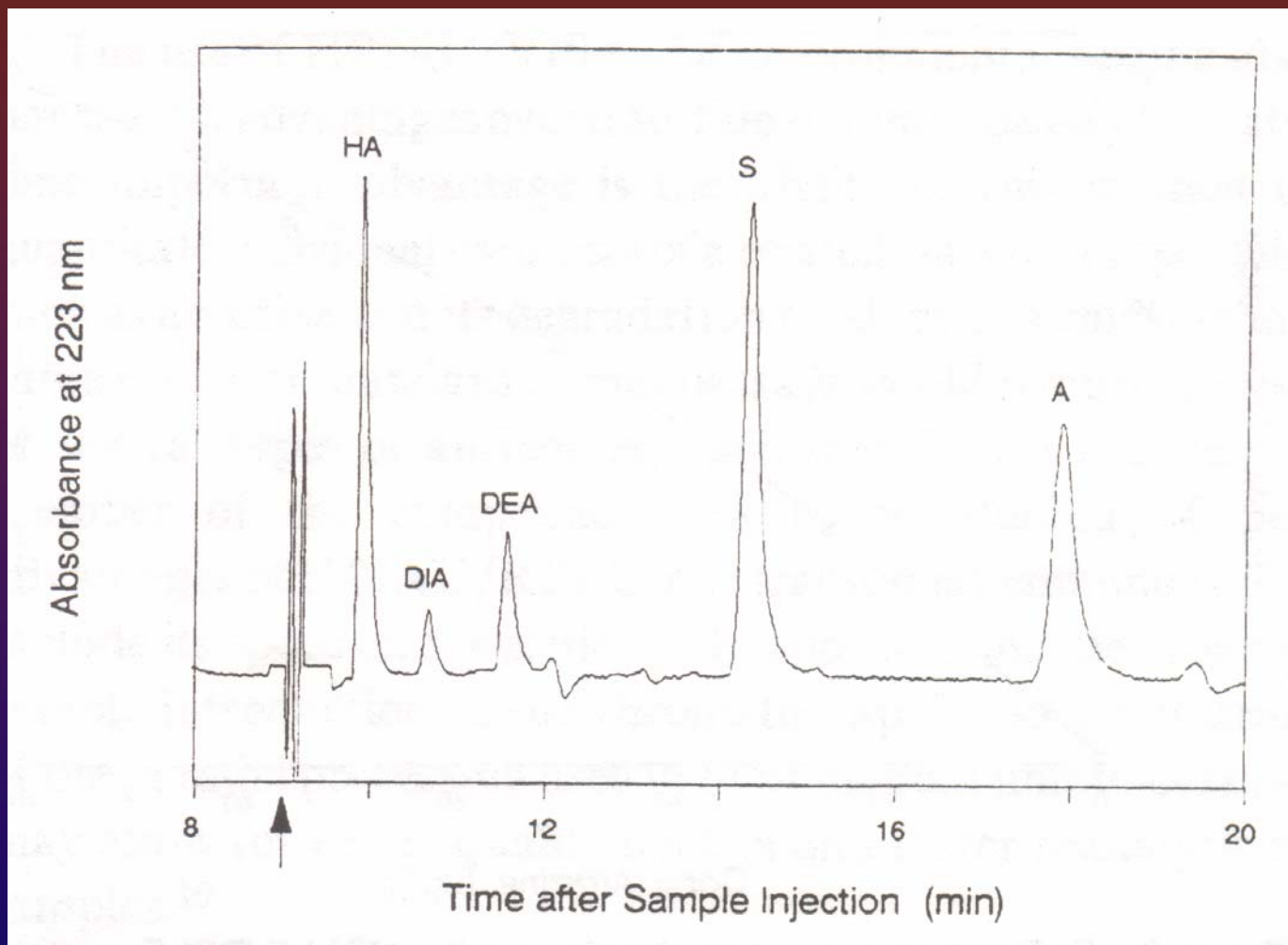
Immobilization of Antibodies to HPLC Supports



The Schiff Base Immobilization Method

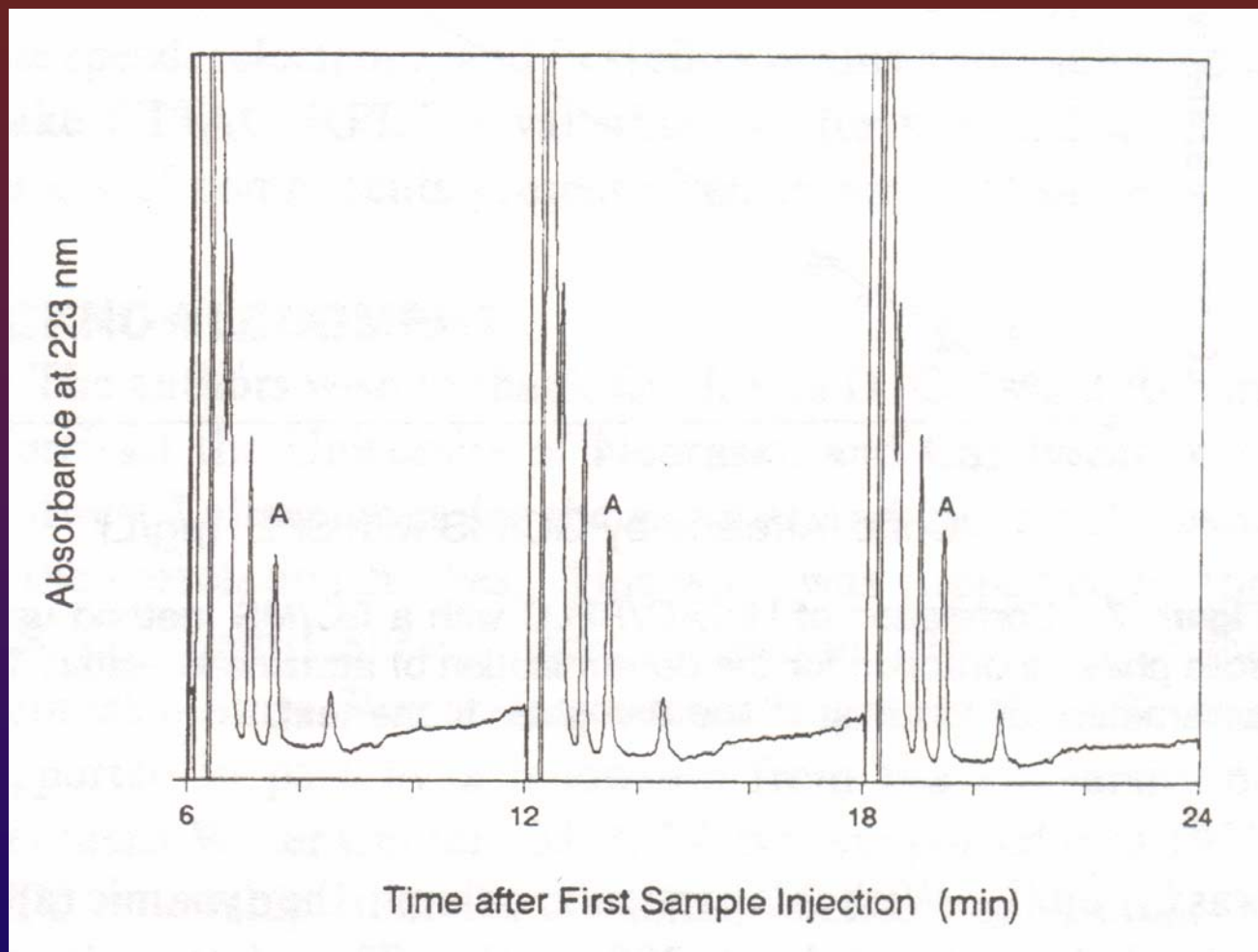
D. S. Hage, J. Chromatogr. B., 768 (2002) 3-30

Analysis of Triazines with a Benchtop Immunoextraction/HPLC System



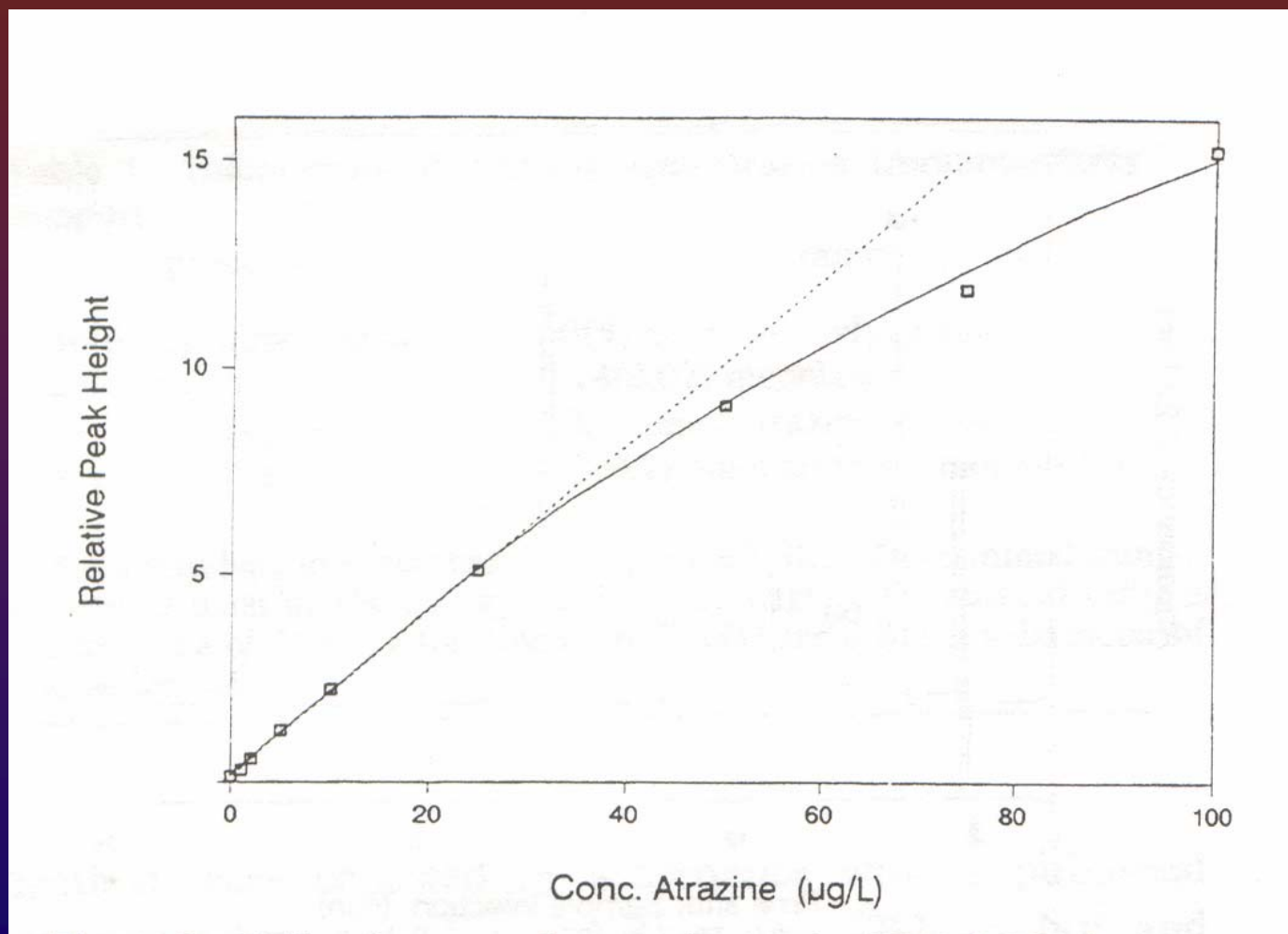
D. Thomas, M. Beck-Westermeyer, D. S. Hage, *Anal. Chem.*, 66 (1994) 3823.

Analysis of Triazines with a Benchtop Immunoextraction/HPLC System



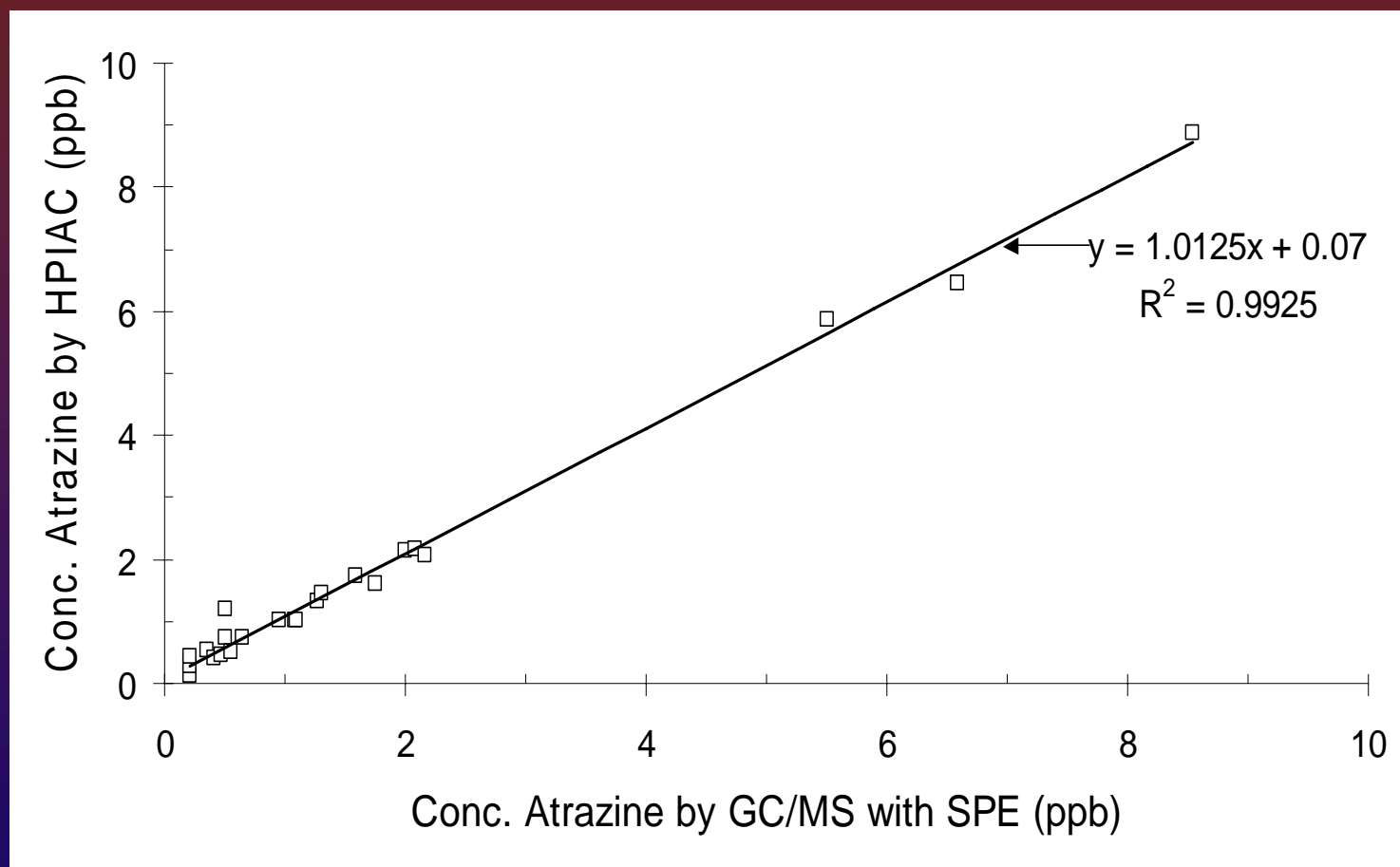
D. Thomas, M. Beck-Westermeyer, D. S. Hage, *Anal. Chem.*, 66 (1994) 3823.

Calibration Curve for the Atrazine by Immunoextraction/RPLC



D. Thomas, M. Beck-Westermeyer, D. S. Hage, *Anal. Chem.*, 66 (1994) 3823.

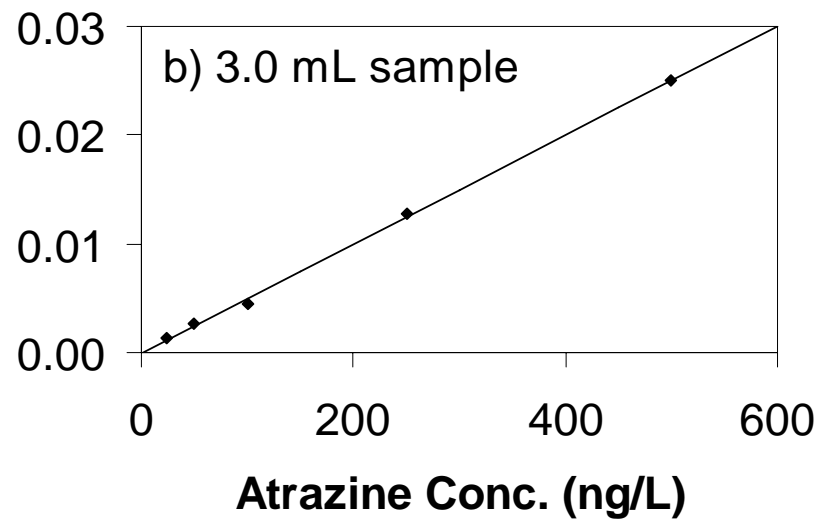
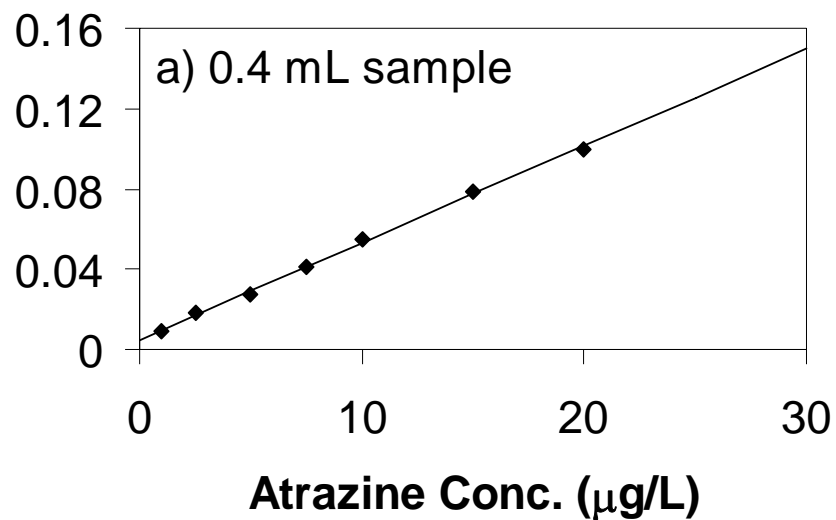
Correlation of Immunoextraction/HPLC to GC/MS



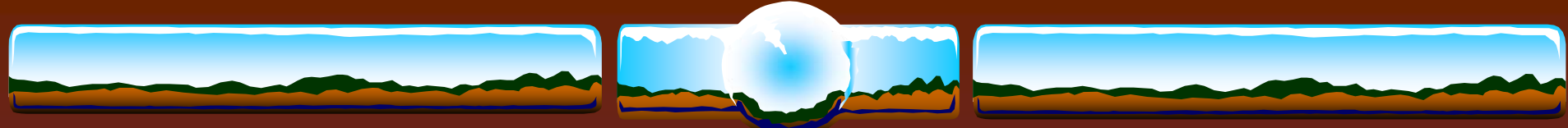
D. Thomas, M. Beck-Westermeyer, D. S. Hage, *Anal. Chem.*, 66 (1994) 3823.



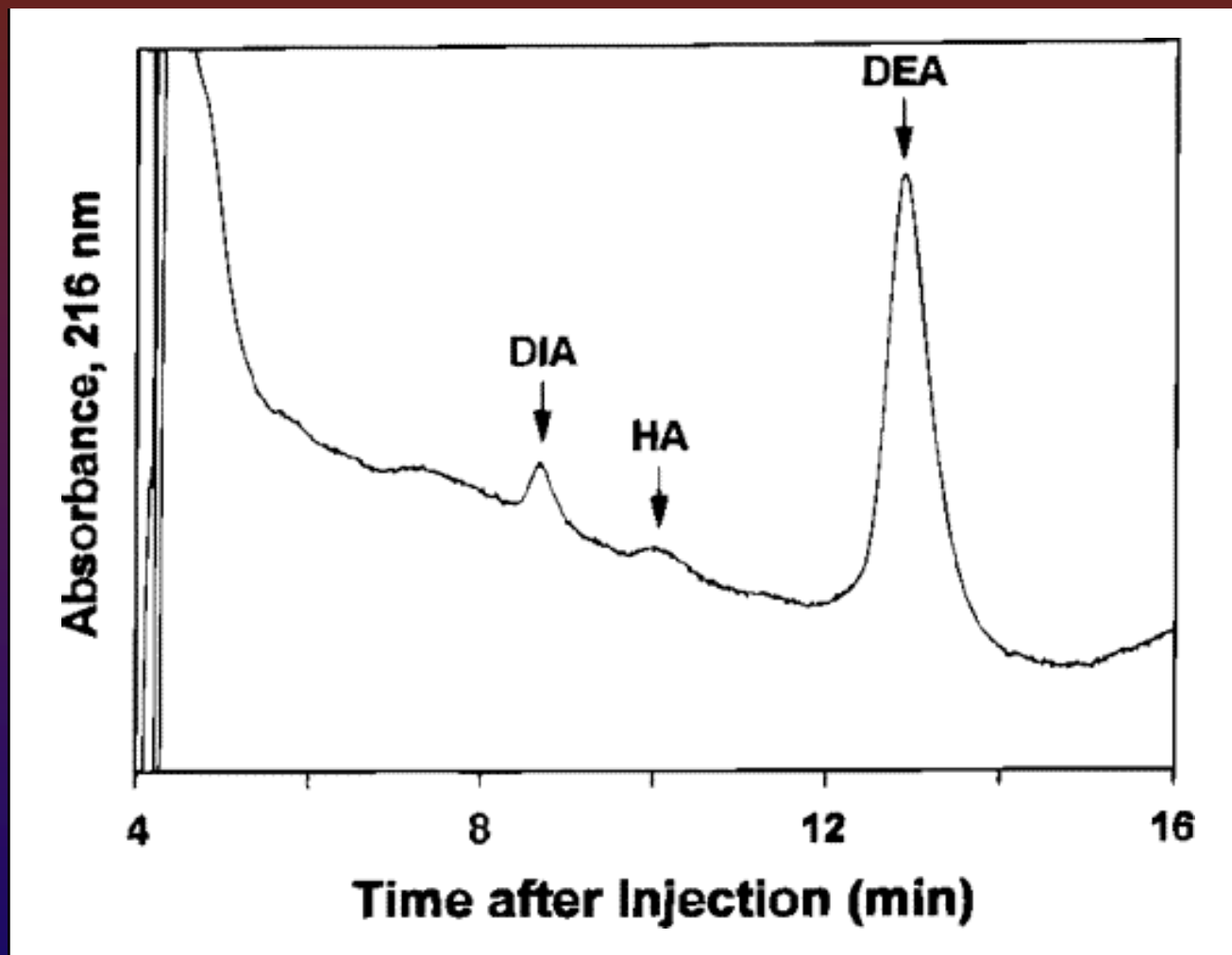
Effect of Sample Volume on Detection Limit



J. G. Rollag., M. Beck-Westermeyer, and D. S. Hage, *Anal. Chem.*, 68 (1996) 3631.

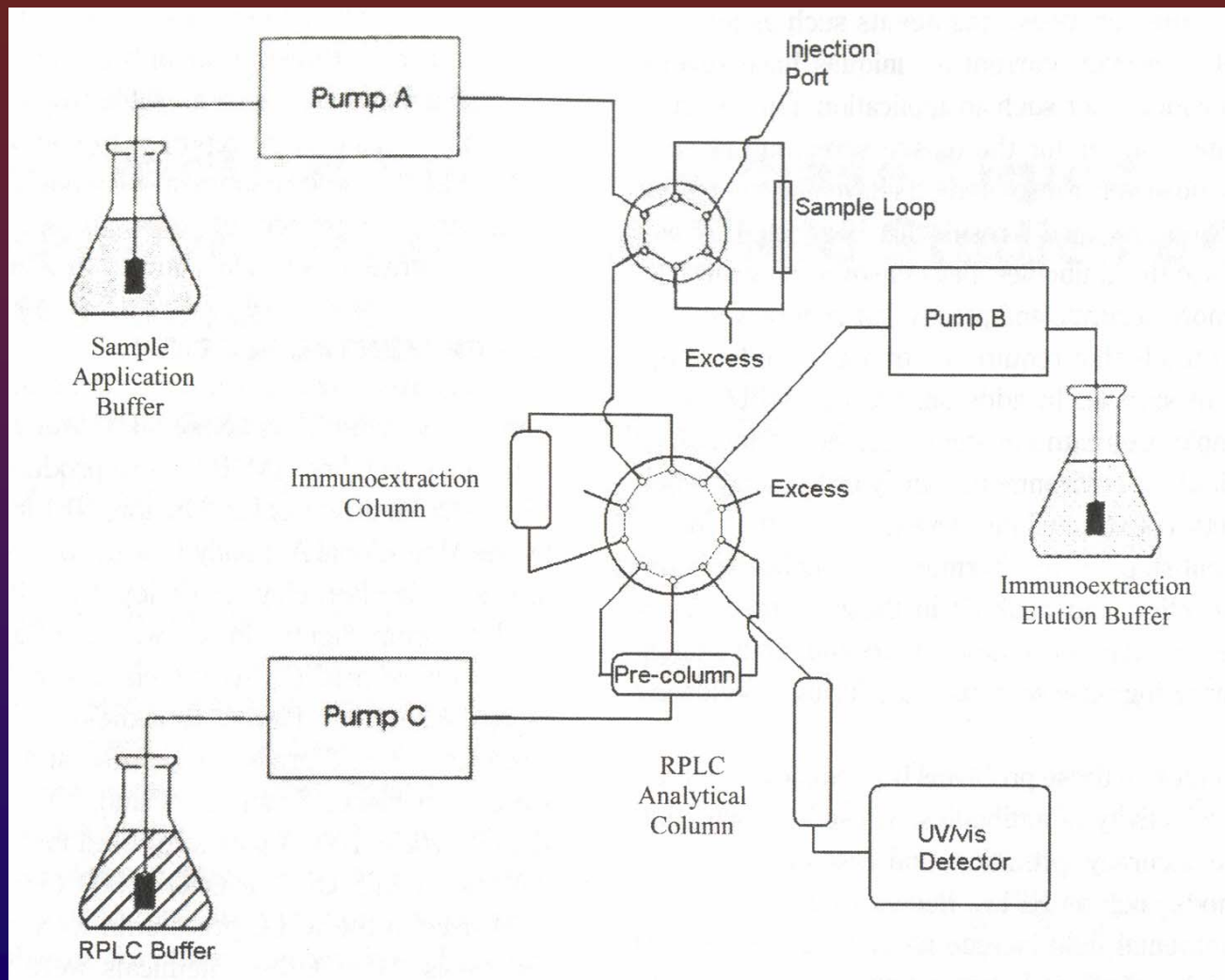


Analysis of Atrazine Degradation Products in a Well Sample from Western Nebraska



J. G. Rollag., M. Beck-Westermeyer, and D. S. Hage, *Anal. Chem.*, 68 (1996) 3631.

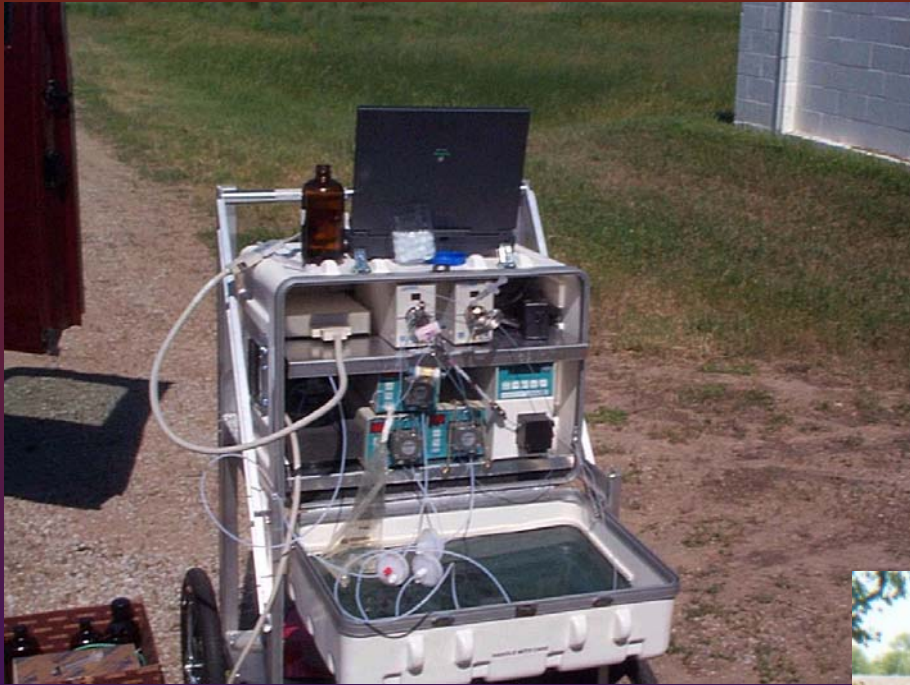
Design of a Field-Portable Analysis System for Immunoextraction/HPLC





Contents of Field-Portable Device

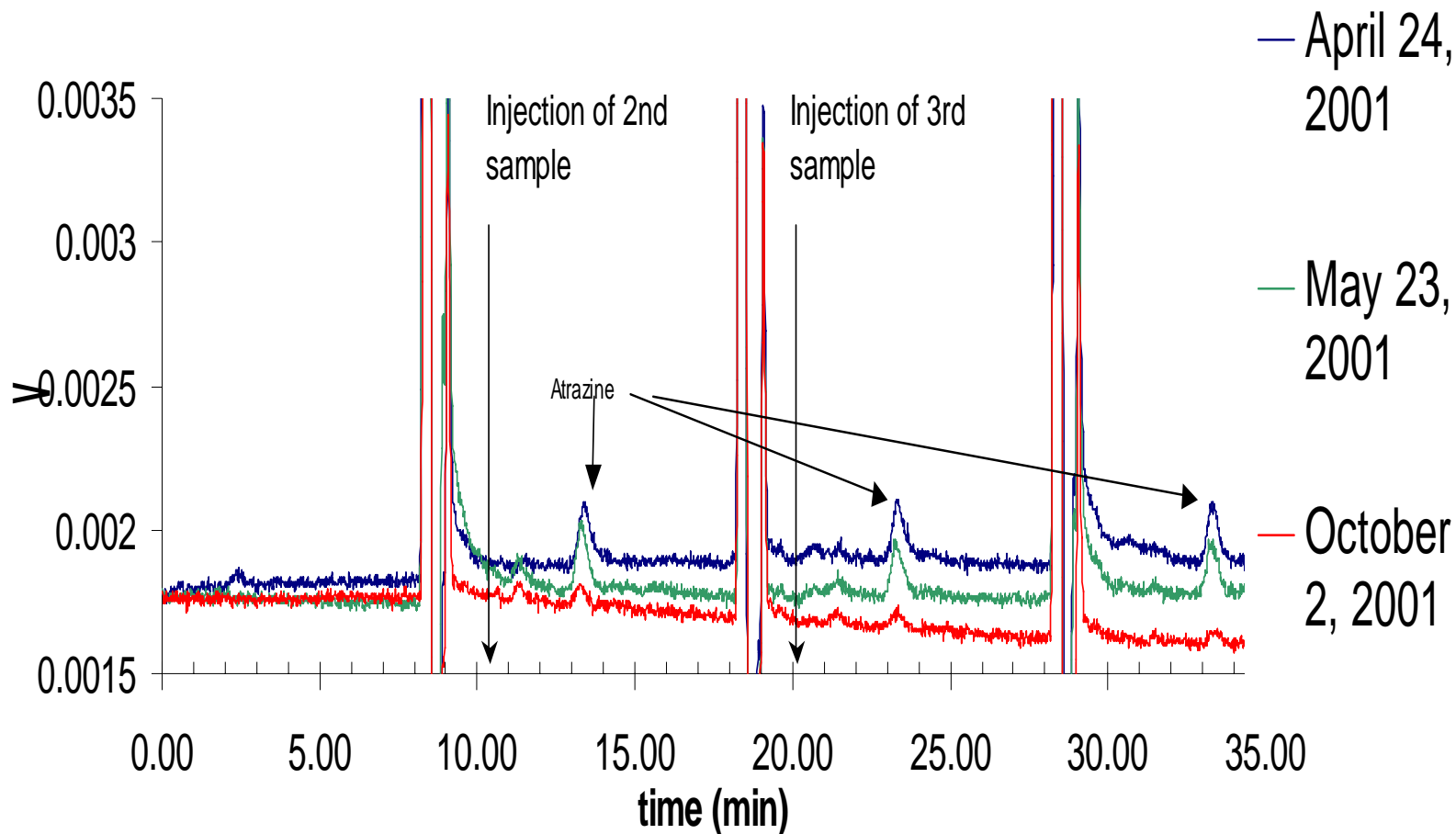
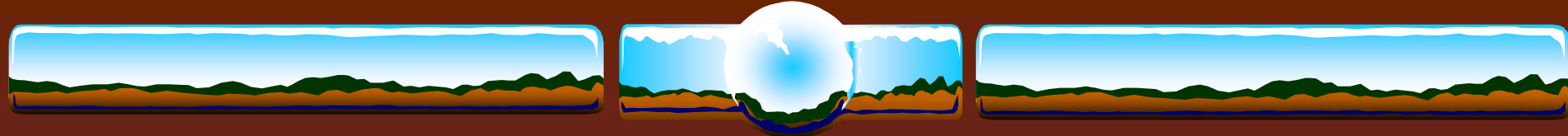
- Rheodyne LabPro valves, one 6-port and one 10 port
- 3 Knauer Micro-Star pump (Sonntek, New Jersey)
- 1 Knauer K-2500 UV/Vis detector (Sonntek, New Jersey)
- Antibody column prepared as previously reported⁵ with Nucleosil silica (Macherey Nagel) and antibodies provided by the University of Nebraska Medical Center (Anti-atrazine) and the Vet Research Council, Czech Republic (Anti-24D)⁶
- Pre column prepared by packing 1 cm x 2.1 mm i.d. column with Pt EPS C₁₈ silica (300A-3 μ m)⁷
- Analytical Column purchased from Alltech: Rocket Column 33mm x 7 mm i.d., (300A-7 μ m) Pt EPS C₁₈ silica packing
- Data Acquisition used programs written by Abhijit Roy Chowdhuri with LabView software (National Instruments, Texas) and PCMCIA DAQ card with SCB-68 shielded interface box



Use of field-portable
immunoextraction/HPLC
system at Lincoln well field
near Ashland, Nebraska

Analysis of water samples
from the Little Arkansas
River in Lawrence, Kansas

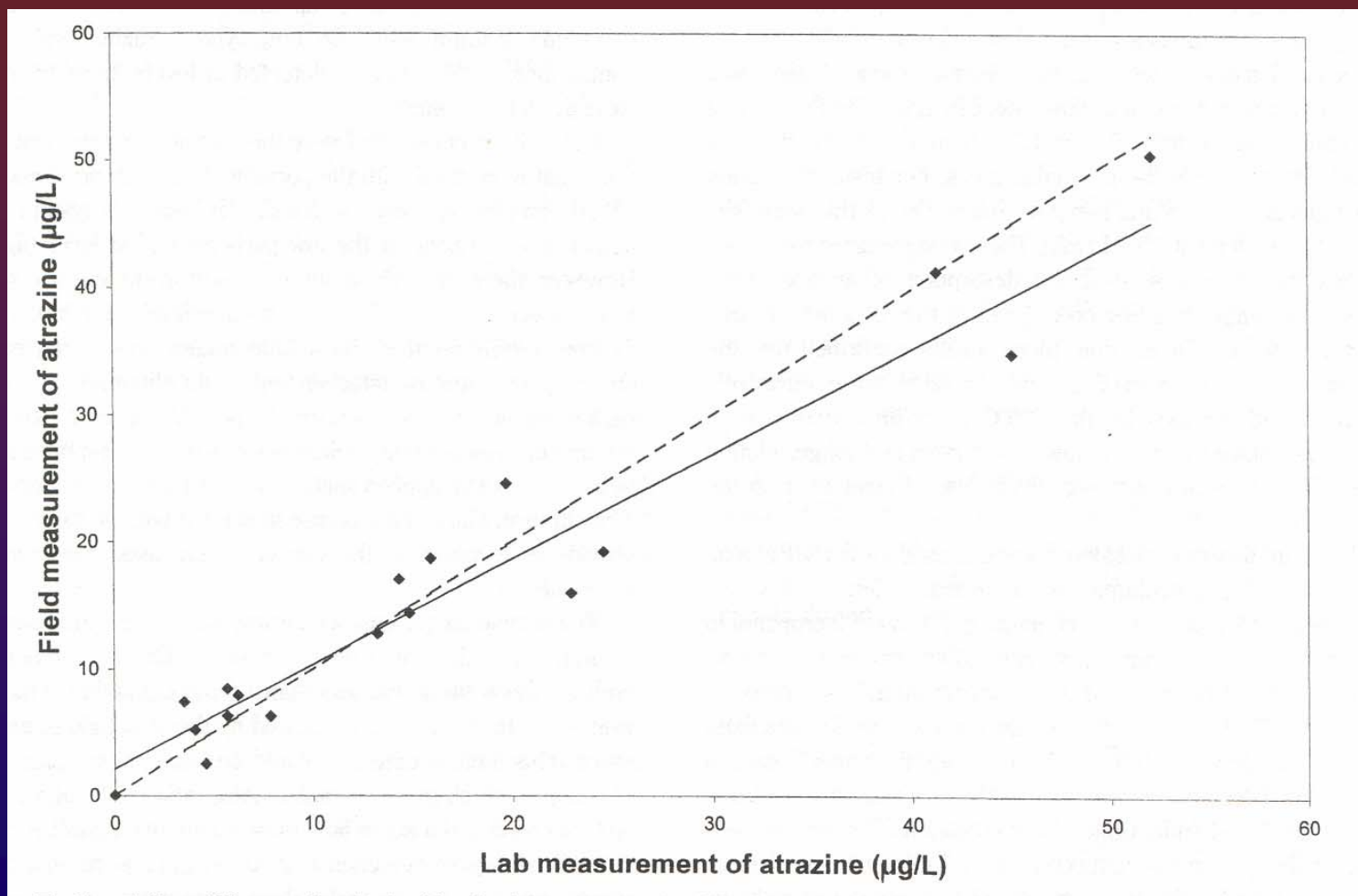




Real time analysis of water samples by the field-portable system

M.A. Nelson et al., Anal. Chem., 76 (2004) 805-813.

Correlation of Lab vs. Field Analysis



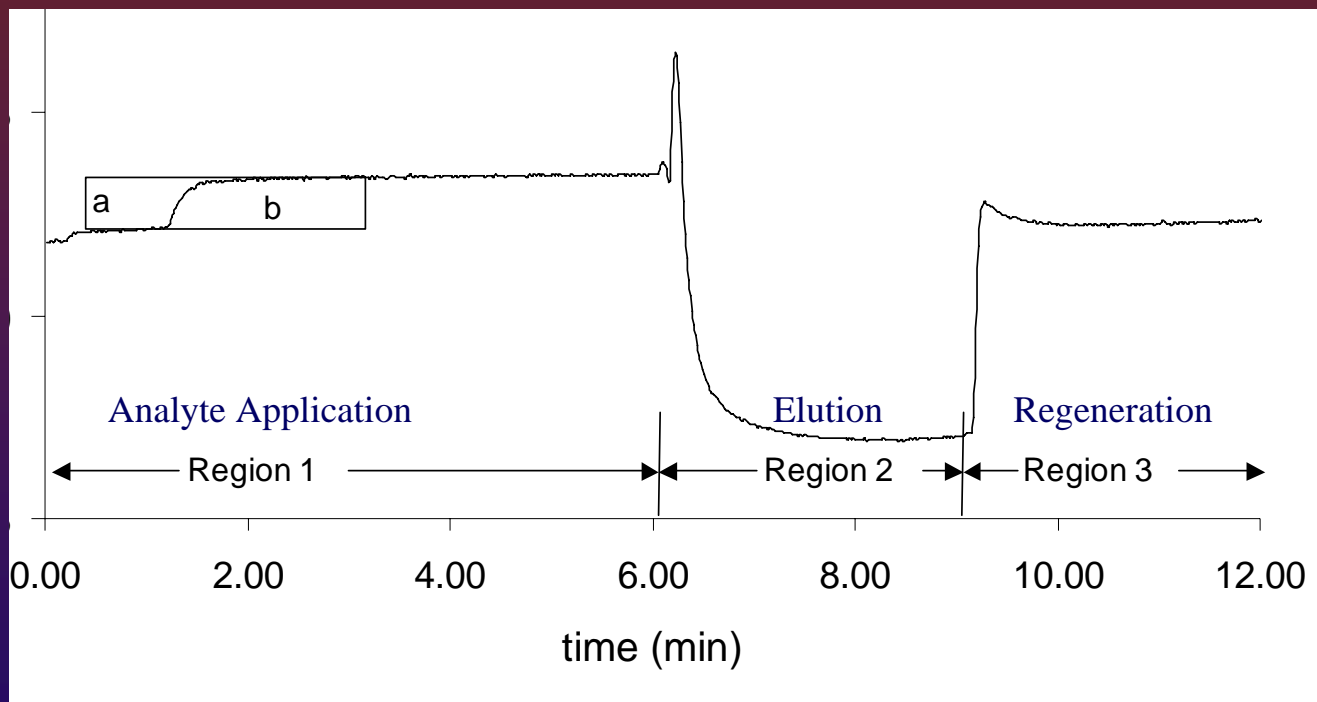
M.A. Nelson et al., Anal. Chem., 76 (2004) 805-813.



Analytes for which immunoaffinity/HPLC assays have been (or are being) developed

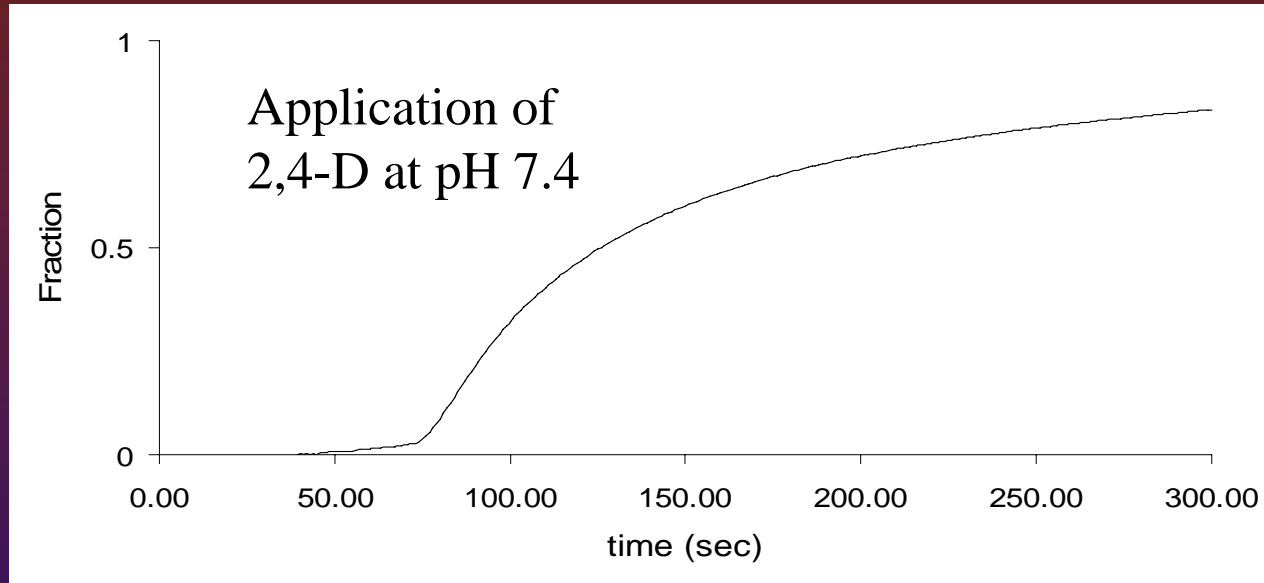
- Atrazine & degradation products*
- Triazine herbicides*
- 2,4-D & chlorophenoxyacetic acid compounds*
- TNT, RDx & related explosive residues
- Other related work
 - Tetracyclines (using aptamers or molecular imprinted polymers)
 - Estrogen mimics (using antibodies or aptamers)

Optimization of Immunoextraction Columns



Application and Elution of 2,4-D on an Anti-2,4-D Column

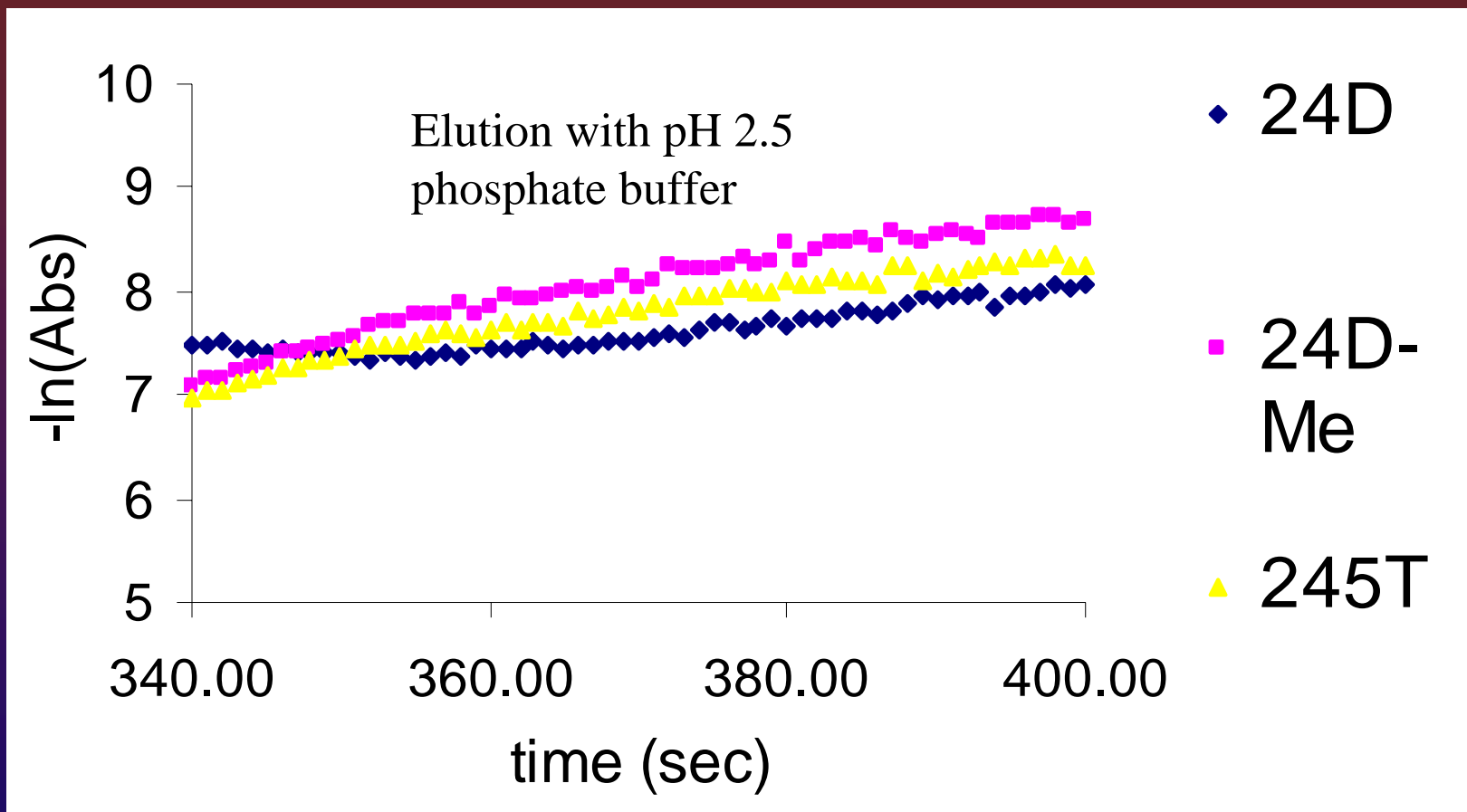
Rate of Analyte Extraction



$$f = \left\{ \frac{S_o}{LoadA} \right\} \cdot \ln \left[1 + \left(e^{\frac{LoadA}{S_o}} - 1 \right) \cdot e^{\frac{-1}{S_o}} \right]$$

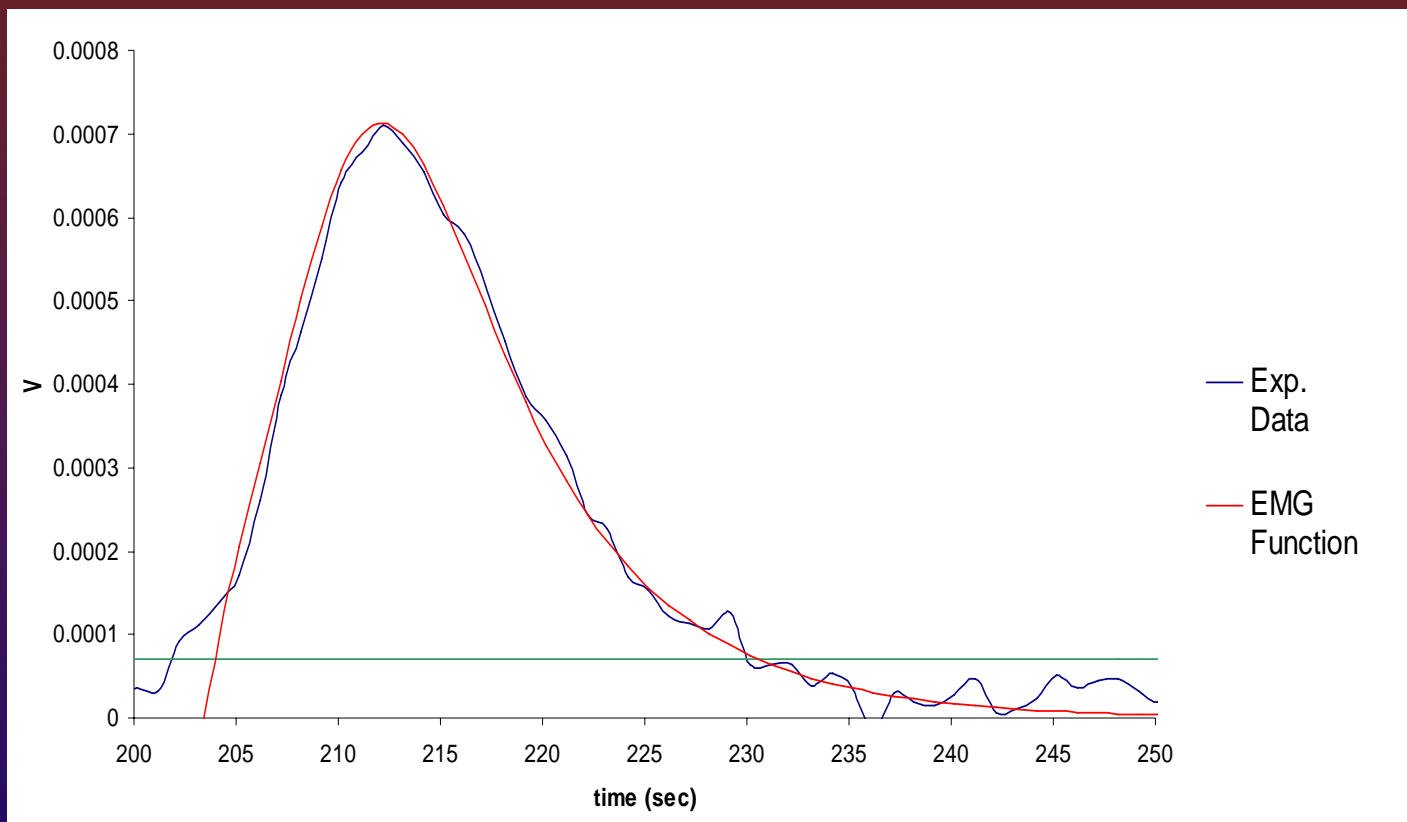
where $S_o = F/(k_A m_L)$, Load A is moles A/ m_L , and m_L is moles of antibody sites in the column, and k_a is the association rate constant

Rate of Analyte Dissociation



$$-\ln [A] = kt - \ln [A_o]$$

Elution of Retained Analyte from RPLC Column



Fit of 2,4-D peak to an exponentially-modified Gaussian (EMG) function



SUMMARY

- On-line immunoextraction/HPLC is a selective technique for the rapid analysis of environmental samples
- This approach combines the specificity of antibodies with the ability to separate groups of related compounds by their polarity.
- A field-portable system based on this approach has been developed for analysis of triazines, with good correlation versus reference methods
- Studies are being performed to allow the rapid optimization of immunoextraction/HPLC for the analysis of other agents, such as 2,4-D, TNT, RDx, tetracyclines, estrogen mimics and related compounds



ACKNOWLEDGEMENTS

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