Steir. Beitr. z. Hydrogeologie / 39 / Seiten 141–147 / Graz 1988

The Identification of Uranine in Natural Water by High Performance Liquid Chromatography (HPLC)

Th. LUTZ & A. PARRIAUX[1])

Der Nachweis von Uranin in natürlichem Wasser durch High Performance Liquid Chromatography (HPLC)

Abstract

The detection of fluorescent tracers in water is often disturbed by the natural fluorecence of the samples. Separation by HPLC allows a sure and rapid identification of the tracer. New Charcoal extraction methods are described.

Zusammenfassung

Der Nachweis von fluoreszierenden Markierungsstoffen in Wasser ist oft durch natürliche Fluoreszenz der Probe gestört. Flüssig-chromatographische Auftrennung (HPLC) erlaubt eine sichere und schnelle Identifikation des Tracers. Neue Extraktionsmethoden für Aktivkohle werden beschrieben.

Résumé

Le dosage des traceurs fluorescents dans les eaux est souvent perturé par la présence d'une fluorescence naturelle. La séparation par chromatographie liquide (HPLC) permet une identification sure et rapide du traceurs. De nouvelles méthodes d'extraction sont décrites.

1. Introduction

The interpretation of tracing experiments with strong uranine coloration rarely presents problems, especially in rapid karstic systems. Tracers are also being used more and more in aquifers of granular porosity, in microfractured mediums or to test secondary diffluences in karstic massifs. In such cases the tracer response comes later and with a smaller intensity. The analytical results of lower concentrations by direct fluorescence spectroscopy must be treated carefully. Sometimes, they have lead to geological surprises, even to unrealistic conclusions. Occasionally, blank samples, especially of charcoal, showed positive signals. This produces a general uncertainty in the experiments and the analytical methods.

The origin of these facts must be sought in the natural fluorescent matters contained in water: mostly organic, anionic substance whose structure cannot be easily defined (humic acids, W. ZIECHMANN, 1980). Here, fluorescence may overlap the tracer spectra (P. L. SMART & I.M.S. LAIDLAW, 1977). The elimination of this phenomenon is difficult with classical methods spectroscopy, modification of pH (H. BEHRENS, 1982) and often impossible with charcoal. Separating the different substances by chromatography is a good solution for this problem (Th. LUTZ, A. PARRIAUX & P. TISSIERES, 1987).

[1]) Laboratory of Geology (GEOLEP), Federal Institute of Technology, Lausanne

High Performance Liquid Chromatography (HPLC) has proved powerful separation method. A large choice of columns and solvents make it very flexible. The new generation of chromatographs guarantees good reproducibility and stability over a long analytical period. This method was chosen by the GEOLEP to increase confidence in their hydrogeological experiments.

2. Equipment

Controlled by a HP-300 Hewlett Packard computer, our LC-4 system from Perkin-Elmer (PE) functions fully automatically. Up to hundred samples may be analysed by coumputer routine and the results are stored on floppy disks.

Computer, chromatograph, autosampler and detector are connected to a network. Pressure, temperature and composition of the mobile phase are controlled and, if necessary, change (gradient elution) by the chromatograph. A second pump is used for post column reactions such as pH correction. The fluorescence detector is a LS-5 from PE. Wavelength, amplification, baseline and other parameters may be optimise during the chromatigramme. Tracers with different spectral characteristics are detectable with their fluorescence maximum (Fig. 1).

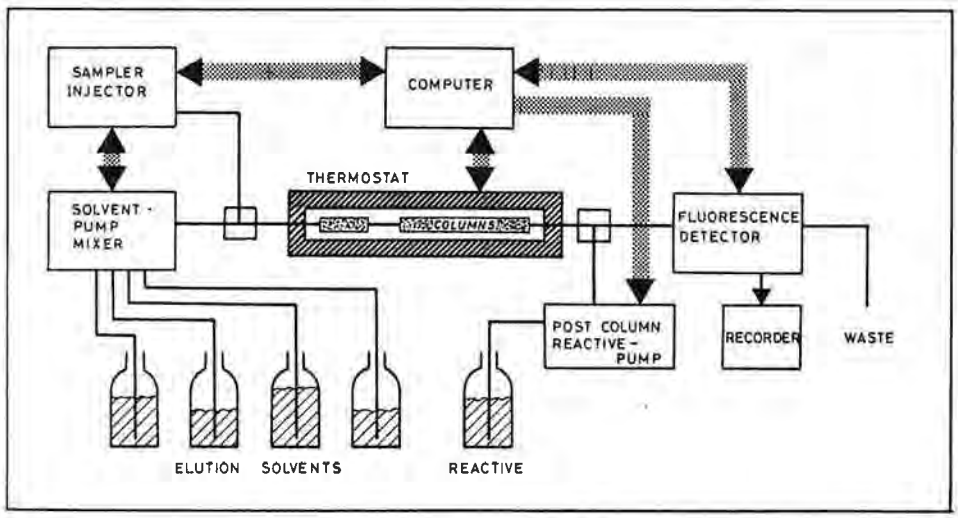

Fig. 1: Analytical system used in GEOLEP.

3. Sample preparation and charcoal extraction

Water samples need no special treatment. Solids must be absent to prevent clogging of the column.

Charcoal (active charcoal as a collector for watertracing) is dryed to guarantee reproducible extraction conditions.

3.1. Extraction of charcoal

3.1.1. Principle

Hydrological tracers are mostly anionic dyes or weak acids. They are fixed on the charcoal by adsorption in non-ionized form. Free acids are much less soluble in water than their salts. To liberate the tracer, classical methods use inorganic bases for ionisation or organic solvents for dissolution. Higher temperature and lower viscosity are favorable for all extraction methods.

An important point is the separation of the sample from solvent. Strong bases or relatively large volumes of solvent may disturb the chromatographie process.

With a volatile extraction medium, the sample may easily concentrated, which permits the reaching of very low limits of detection.

3.2. Classical methods

3.2.1. Inorganic bases

Extraction with potassium-hydroxide in ethylalcohol shows important defects. The mixture is not stable and colours quickly (F. BAUER, 1967). The light absorption rises so that detection of the tracer becomes impossible. This effect may be so strong that uranine signals have been found even in fresh charcoal. Last but not least, the solvent is dangerous for eyes, skin and spectrometers.

3.2.2. Organic solvents

There are many possible organic solvents. Dimethylformamide is very efficient to extract tracer dyes from charcoal (G. ACKERMANN, F.P. BUB & H. HÖTZL, 1982). Its price and toxicity are relatively high. This product is not volatile. Hence, it is impossible to concentrate the sample or to recycle the solvent.

3.3. New extraction methods

3.3.1. Inorganic bases

Experiments at GEOLEP have shown that a mixture of lithium hydroxide in methyl- or ethylalcohol is stable over some weeks and efficient in extraction, but still dangerous. A good alternative is ammonia in alcohol (10% of conc. NH_4OH). Uranine is completely extracted and the solvent is less toxic (J.-D. AUBORT & A. ETOURNAUD, 1984). Theoretically, it evaporates without residue. In practice, more or less ammonium carbonate is formed, an undesired buffer substance in the chromatographic process. This extraction medium can be used for direct spectroscopie analysis.

3.3.2. Organic bases

The characteristics of a good extraction medium are: high ionisation capacity for the tracer and excellent solvent for the ion. It must be volatile, stable, miscible with water, nontoxic, and have a low price. Tested bases like ethylamine, propylamine, aniline and others do not offer these properties. This is not the case of pyridine. Its ionisation capacity for fluoresceine (free acid of uranine) is so high that 10% in water extracts

the dye completely from charcoal. Water may be replaced by methyl or ethylalcohol or acetone without disadvantage. The range for the pyridine concentration is useful from 5 to 100%.

All these mixtures are stable over some months and show no change in light absorption even in the UV range.

The toxicity of pyridine both for men and the environment is not a problem.

3.4. Practical extraction

For low level detection of fluorescent tracers, the best way to prevent contamination is an easy sample preparation. Our method is as follows:

In an open 5 ml plastic styring 1 cm of cotton wool comes first, then 2 ml (1 g) of charcoal. With the piston, 2 ml of solvent are aspirated and the styringe is closed with a capsule.

After 15 min. or more, the extraction is complete and the solution is directly transferred to a simple flask of the chromatograph. If no analysis follows, the solvent is evaporated at 50° C. The residue is stable, even for later controls.

4. The chromatographical separation

4.1. General description

This analytical method is valid for water samples and charcoal extracts. To separate the fluorescent dye from the humic acids, differences in solubility and ionisation may be used (R. W. YOST, L. S. ETTLRE & R. D. COULON, 1980).

The detection is made by fluorescence at the optimal wavelength for the tracer used. The amplification of the spectrometer may be high, and the slits large, because the dye is measured in pure solvent with a low background fluorescence.

4.2. Reverse phase chromatography

4.2.1. Principle

The chromatographic column contains as stationary phase a silica-gel with nonpolar groups on its surface (aliphatic C_{18}). The eluent (mobile phase) is more or less polar, like water, alcohol or acetonitrile. If UV cutoff is not a problem, acetone may be used as well. The solubility of unionized dye is better in the stationary phase than in the eluent. It is retained. Each substance has its own solubility in these two phases. The retention time is directly dependent on solubility and separation results.

4.2.2. Uranine and humic acids

Uranine and humic acids are separated on a reverse phase column under the following conditions.

Dry charcoal extracts are dissolved in methanol, water samples are directly injected.

Eluent	95 parts formic acid 0.1% 5 parts methanol pH < 5
Flow	2 ml/min.
Column	12 cm 4.5 mm Partisil C_{18}
Post column	10 parts Ammonia 10%
Detector	fluorescence 490/515 nm slits 10/10

The dye and the humic substances are free acids in this eluent. The chromatogramme (Fig. 2) shows a poor separation of each humic acid, but a good separation from the tracer. If high flow rates are wanted, the methanol which increases the viscosity of the eluent must be replaced by acetonitrile or acetone. Price and toxicity defend the use of acetone, separation characteristics the use of acentonitrile.

The problem is the detection. At this pH (< 5) uranine gives no fluorescence. With a second pump ammonia is added as post column reactive to raise the pH.

Apart from the relatively high investment for hardware (2 pumps), the reverse phase chromatography is reliable and reproducible.

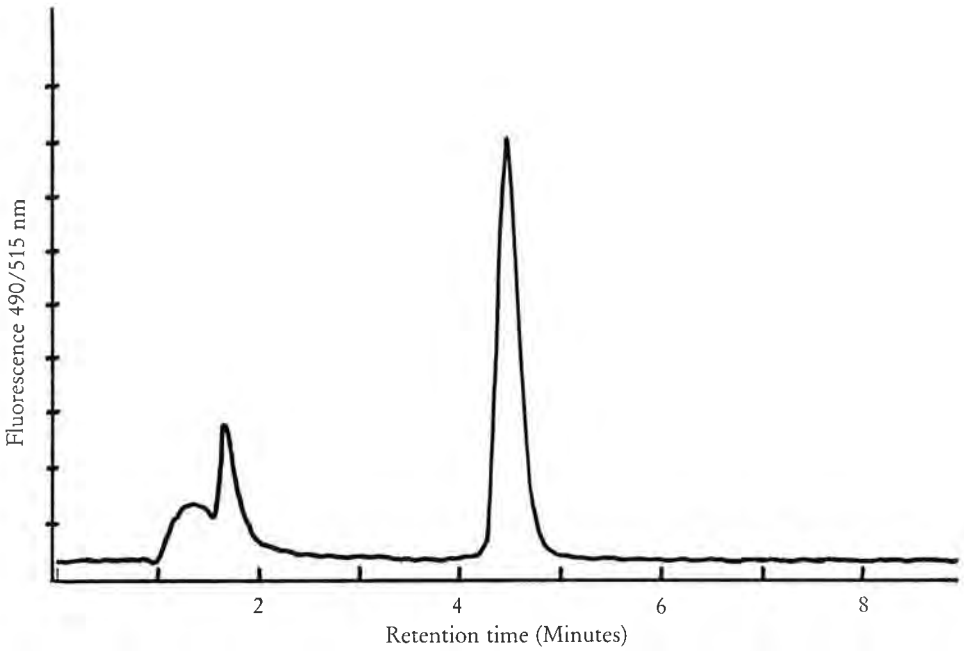

Fig. 2: Sample Spring Fontaney (coord. 565,06/129,74/515). Charcoal, 28. 5. 84–4. 7. 84, extracted with alcohol/ammonia 10%. Reverse phase chromatogramme, conditions: see text. Peak at 4.5 min = uranine.

4.3. Ion exchange chromatography

4.3.1. Principle

Most tracers and humic substances are more or less strong acids. This allows separation on anion exchange columns (D.T. GJERDE & J.S. FRITZ, 1987). As stationary phase, tertiary amino groups on silica are used. (Resins don't support all solvents.) The pH and ion concentrations are varied to find the optimal separation.

4.3.2. Uranine

To guarantee pH stability, the eluent is a buffer solution such as phosphate or citrate. An organic solvent is also added to wash out high molecular humic substance or non-ionized components.

Eluent	90 parts citric acid 5mmol, pH 5,5 10 parts acetone
Flow	4 ml/min.
Column	15 cm 4.5 mm Waters SAX
Post column	5 parts NH_4OH 1%
Detector	fluorescence 490/515 nm slits 10/10

4.4. Ion pair chromatography

Ion pair chromatography is a special kind of ion exchange chromatography. An eluent with a dissolved ion exchanger, for instance triethanolamine, flows on a normal C_{18} reverse phase column. The aminogroup reacts as an anion exchanger. The aliphatic tail fixes on the column. The separation effect is similar to ion exchange. An important advantage of this method is the relatively high pH while separation remains good. Uranine is directly detectable. C_{18} columns are much cheaper than ion exchangers. The disadvantage is that a C_{18} column used with an ion-pair never can work again in reverse phase. (Ion-pair substances are so surface active that they cannot be washed out.)

Another point is the high sensitivity of the stationary phase to changes in the eluent or sample.

To compare standards with samples, ion concentration and pH must be identical. A good way to identify the tracer pic is standard addition. Chromatogrammes of the pure sample and of a sample mixed with tracer are compared.

The development of optimal separation parameters in ion chromatography is often long and difficult and justified only for frequently used tracers.

4.5. Pyridine method

4.5.1. Principle

From the described methods, reverse phase is the most flexible and easiest separation system. The disadvantage is the low pH of the eluents. Full ionized tracers or humic acid are not retained and not separable unless strong cations like sodium, potassium or ammonium control the pH. Pyridine as a cation seems to ionize less strongly, so that separation becomes possible. Details of this phenomenon are the subject of research.

4.5.2. Uranine

Charcoal extracts are dissolved in 1% pyridine or, if not dried, directly used like the water samples. The retention time of uranine is controlled by the eluent and is not sensitive to sample composition as shown in figures 3 and 4.

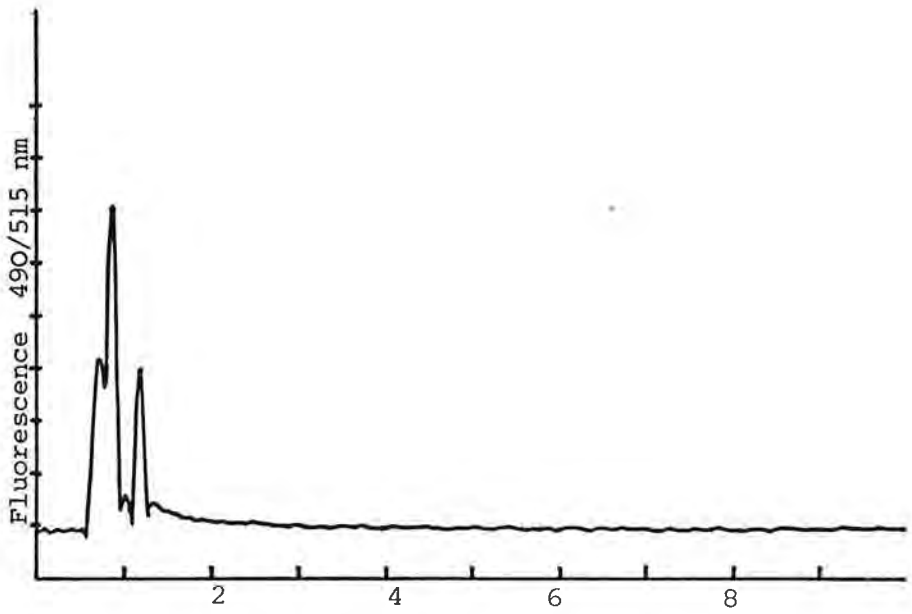

Fig. 3: Sample Spring Cornallaz (ccord. 547,42/149,24/597). Charcoal 25.10.85 – 11.2.86.

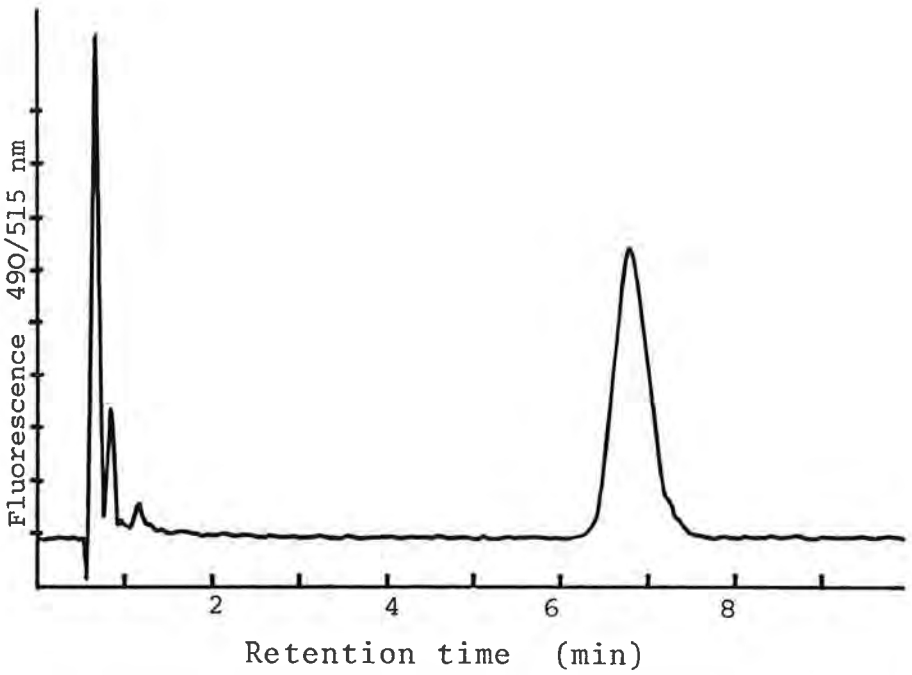

Fig. 4: Sample Spring Cheseaux. Charcoal 24.10.85 – 11.2.86. Extraction with pyridine 10 % in H_2O. Reverse phase chromatogramme, conditions see text. Peak at 7 min = uranine, 15 pg.

Eluent	80 parts pyridine 1% in water 15 parts formic acid 0,1% 5 parts acetone
Flow	4 ml/min.
Column	30 cm 4.5 mm Partisil C_{18}
Post column	none
Detector	fluorescence 490/515 nm slits 10/10

The composition of the eluent may be varied in a wide range to optimize separation for other dyes or samples.

Excellent longterm stability of the retention time was found.

5. Perspective

HPLC resolves the problem of interference in the identification of uranine at low concentrations and in complex matrices.

The method is routine in our laboratory. The simultaneous separation of several different tracers has been realized on standard samples.

Field experiments are in progress and we are working to develop routine methods for multitracing.

6. Conclusions

Geologists need a tool for a sure uranine identification. HPLC is complex and expensive in hardware and know-how. For low level detection of hydrological tracers this is the method of choice. The relatively high investment is justified by the possibility of automation to save manpower and to gain reproducibility.

References

ACKERMANN, G., F.P. BUB & H. HÖTZL (1982): Erfahrung mit der Dünnschichtchromatographie beim Nachweis von Fluoreszenztracern.- Beiträge zur Geologie der Schweiz, Hydrologie,**28-I**, 63-68, Bern.

AUBORT, J.-D. & A. ETOURNAUD (1984): Recherche et dosage de trace de fluorescine dans les essais de coloration en hydrogologie.- Trav. chim. alimnt. hyg.,**75**, 415-420.

BAUER, F. (1967): Uraninnachweis mit Aktivkohle.- Steir. Beitr. z. Hydrogeologie, **18/19**, 169-178, Graz.

BEHRENS, H. (1982): Verfahren zum qualitativen und quantitativen Nachweis von nebeneinander vorliegenden Fluoreszenztracern. Beiträge zur Geologie der Schweiz, Hydrologie, **28-I**, 39-50, Bern.

GJERDE, D. T. & J. S. FRITZ (1987): Ion Chromatography.- 2nd Ed., Hüthig Verlag, Heidelberg.

LUTZ, Th., A. PARRIAUX & P. TISSIERES (1987): Tracage au Gouffre du Chevrier (Pralpes vaudoises) et methode d'identification de l'uranine faible concentration.- Bulletin du Centre d'Hydrogeologie de l'Universit de Neuchatel, 7, 1987.

SMART, P. L. & I. M. S. LAIDLAW (1977): An Evaluation of some fluorescent Dyes for Water Tracing.- Water Resource Research, **13/1**, 15-33.

YOST, R. W., L. S. ETTRE & R. D. COULON (1980): Practical Liquid Chromatography.- Perkin-Elmer.

ZIECHMANN, W. (1980): Huminstoffe.- Verlag Chemie, Weinheim.