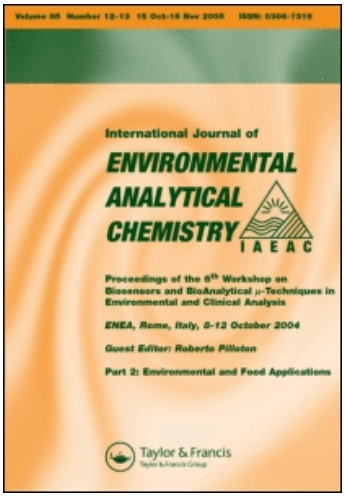

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Isolation of Organic Compounds from Water for Chemical Analysis and Toxicological Testing†

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KEY WORDS: XAD-isolation, XAD-cleaning, automation, Mutagenicity

In most investigations on water quality it is not possible to determine the relationship between toxicological and chemical parameters as a consequence of different isolation and sample preparation techniques. A combined research made by the analytical-chemical and biological section of KIWA has led to the development of a single isolation and sample preparation method for organic micro-pollutants in water, suitable for both toxicological and chemical measurements as the Ames test, group parameters (OCl, ON, OS, OP), GC-MS and H.P.L.C.

An automated installation for the adsorption of volatiles to Tenax and of slightly-volatile and non-volatile compounds at pH 7,2 and 9 to XAD-4 has been built.

Following the adsorption the volatiles are thermically desorbed from the Tenax. The XAD columns are eluted with ethanol and an ethanol-cyclohexane mixture. The eluates are dried and concentrated by means of an azeotropic distillation. This procedure has been automated as well.

GENERAL

The necessity to analyse water arose from the moment that it was supposed or known that undesired and even harmful organic compounds

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are present in surface and groundwater, or may be produced during water treatment. The question "What is waterquality?" has to be answered as simple and quick as possible. With a suitable analytical chemical programme a large number of compounds can be measured routinely. In that way the quality of a specific watersample can be monitored as far as its quality is not affected by micro-pollutants not covered by the analytical programme. But also with respect to detectable compounds, we have to take into account that a measured concentration does not provide any information about for example toxicity or taste of a watersample because there is little or no knowledge about additive, synergistic and antagonistic effects of organic compounds. Toxicological tests provide information about possible toxicity of a polluted watersample, however they do not provide any information about the identity of the compounds responsible for the toxic effect observed. Only if the results of the chemical and toxicological measurements can be related to each other, an optimal information is obtained on the character and the risks of waterpollutants.

The necessity of a direct relation between chemical and toxicological measurements implies that the various techniques have to be carried out in one and the same sample.

Since concentrations of organic micro-pollutants in water are very low most of the time, we have to isolate and concentrate these compounds. This means that the same isolation and sample preparation procedure has to be performed for both toxicological and chemical investigations. Both of these techniques have their own strict requirements with regard to the composition of the isolated and concentrated organic material.

These requirements are:

— for toxicological test (in the first place the salmonella microsomal mutagenicity test or Ames test): the isolated organic material has to be present in a watersoluble solvent that is neither toxic nor mutagenic.
— for the different group-parameters: the solvent has to be free of chlorine, sulphur, nitrogen and phosphorus.
— for liquid chromatography: the solvent should not show any absorption at the wave-lengths used for the detection.
— for gas chromatography and mass-spectrometry we need water-free samples. For the concentration of an organic solution by evaporation the solvent has to be free of water as well as segregation and precipitation may occur with water present in the concentrate.

The more commonly used techniques for the isolation of organics from water are

— gas-stripping and adsorption on Tenax[1]
— solvent extraction[2,3]

— carbon absorption[4]
— freeze drying[5]
and XAD adsorption.[6,7,8,9,10,11,12]

In particular the isolation of organic compounds with XAD has proved to be a very useful technique. Recovery efficiencies have been reported a.o. by Junk,[6] van Rossum,[7] Osterroht,[13] Tateda *et al*,[14] Renberg,[15] Nikaido *et al*,[16] and Jones.[17] It has been shown that pH adjustment has to be carried out to obtain good recovery for the different types of compounds.

However, none of the published methods is satisfactory suitable for both chemical as well as biological analysis, because they were not developed with that intention.

A combined research programme of the analytical chemical and biological section of KIWA has led to the development of a single isolation and sample preparation method for organic micro-pollutants in water (Fig. 1).

This method was developed according to the requirements mentioned above so that both the Ames test and the different chemical techniques like the group parameters OCl, ON, OS and OP, GC-MS and HPLC can be performed in the same concentrate.

The procedure is a combination of a gas stripping technique for the isolation of volatiles and a number of adsorption steps whereby the watersample is carried through the macro-reticular resin XAD-4. The organics which are passing the XAD can be isolated from the XAD-effluent by means of freeze drying concentration.

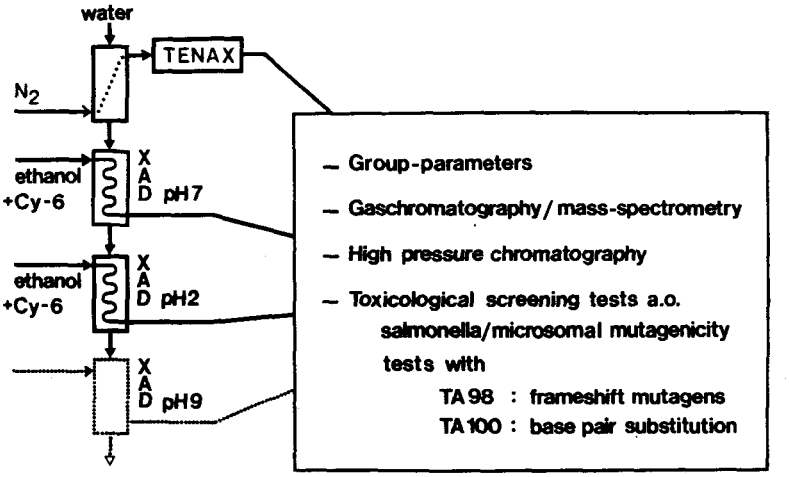

Figure 1 Isolation and sample preparation for chemical and toxicological analysis.

ISOLATION

The isolation process is based on a competition between the surrounding water and the isolating medium XAD. This competition is strongly affected by the composition of the watersample. Since the organism of the consumer of drinking water "contacts" the water at different pH values, namely at pH 7 in the mouth and the gullet, at pH 2 in the stomach and at pH 8.7 to 9 in the intestines, there is a reason to carry out the isolation at those three pH values.

Depending on the type of water 60 to 300 liters of water are required for a complete programme including the Ames test and the various chemical measurements.

The set-up of the isolation apparatus is shown in Figure 2. Water is supplied continuously to an overflow flask with a constant level *a*. The

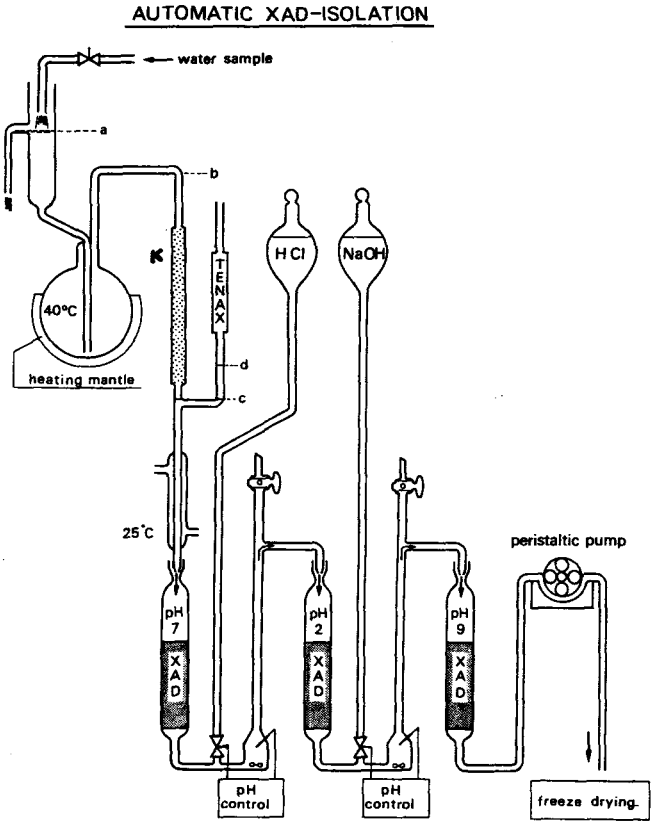

FIGURE 2 Automatic XAD-isolation at pH 7,2 and 9.

water is heated to 40°C and then enters at *b* a column *K*, which is filled with Rashigrings. In this column dissolved gasses are removed from the water.

As it can be considered as an air-column the difference in water level between *c* and *d* will be equal to that between *a* and *b*. When the air-column in *K* increases in volume because of degassing, level *c* will go down and the gas-mixture will escape through the Tenax-column, where the volatile organic compounds can be adsorbed.

After cooling down to 25°C the water passes a number of XAD-columns at pH 7,2 and 9 at a flow rate of one bed-volume per minute. This flow rate is controlled by a peristaltic pump at the outlet of the apparatus.

The quantity of resin used in each isolation-step is one milliliter per liter of water. That implies that a complete isolation-procedure, independent of the sample size, requires 1,000 minutes, or nearly 17 hours, and can be done largely overnight. The installation has been devised in such a way that it can be used in a laboratory as well as at various locations in production plants for drinking water.

Figure 3 shows a classification of the isolated compounds, according to their chemical and physical properties as far as they are identified with GC-MS and HPLC.

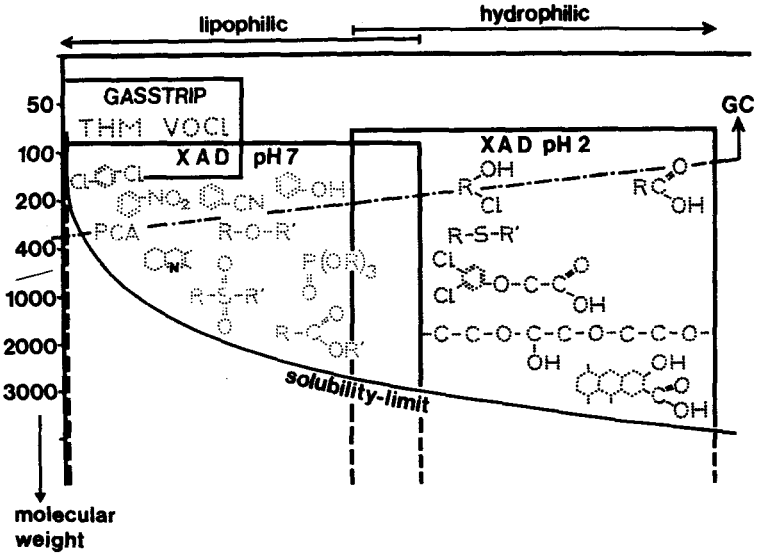

FIGURE 3 Classification of isolated compounds according to their chemical and physical properties.

With the gas stripping technique lipophilic volatiles are isolated with a molecular weight of from 30 up to about 150. At pH 7 XAD adsorbs undissociated compounds with at most one weak polar substitution and with a molecular weight lower than 2,000. At pH 2 compounds are isolated which can be adsorbed on the XAD because they have been associated as a consequence of the lowered pH.

These are weakly hydrophilic, acid reacting compounds with one strong polar substitution or a number of weak polar substitutions and with a molecular weight up to 3,000.

TABLE I

Recoveries of XAD-4 isolation at different pH values, analysed with G.C. (– = not measured)

Compound	Concentrations µg/l drinking water	pH			
		2	7	9	12
Fluoranthene	28	89	88	—	96
Acetophenone	68	68	73	—	63
Heptanol-1	58	89	83	100	100
Trichloroethylphosphate	83	80	81	—	66
2,4-Dichloroaniline	56	86	85	—	90
2,3-Dichloroaniline	64	84	91	—	85
3,4-Dichloroaniline	55	39	95	88	89
2,6-Dichloroaniline	67	84	91	—	88
2-Methyl, 4-nitroaniline	77	73	90	—	100
Tributylamine	53	0	81	87	79
Dibenzylamine	56	5	94	—	97
Dodecylamine	88	0	25	—	42
2,2,6,6-Tetramethylpiperidine	62	0	0	—	73
Dibutylphthalate	32	89	100	—	9
3,5-Dichlorophenol	96	98	85	—	1
Dichlorophenoxyaceticacid	12	>70	0	—	—
p-Nitrophenol	127	47	3	—	0

The same process will occur with the basic reacting compounds at higher pH-values. This isolation step is still in the development stage. Only a small part of the isolated organic material (about 5%) can be analysed by GC-MS. The greater part has a high molecular weight and has to be analysed with other techniques such as HPLC. Recovery measurements have pointed out that lipophilic compounds can be isolated for 80 to 100 percent at pH 7. The recoveries for the weakly hydrophilic compounds at pH 2 are between 50 and 100 percent. (Table I).

ELUTION

The volatile compounds adsorbed to the Tenax-column can be analysed after thermal desorption. The desorbed compounds can be tested in the Ames test. However, the composition of this group of compounds is not very complex and can be easily analysed by GC-MS. This technique is to be preferred over the time consuming Ames test.

The XAD columns are dried with nitrogen and then eluted with 5 bed-volumes of ethanol and subsequently with 5 bed-volumes of a mixture of ethanol and cyclohexane in order to achieve an optimal complete elution of both polar and non-polar compounds. The first elution with ethanol will also remove any remaining water trapped in the pores of the resin. In this way the non-polar elution solvents will also be able to enter into the pores of the resin.

The XAD columns are eluted at a flowrate of 0.1 bed-volume per minute after an equilibration time of 15 minutes.

If the total organic chlorine parameter has to be measured in the eluate, the neutral and acid XAD columns are washed before the elution with a solution of sodium nitrate and nitric acid respectively to remove remaining inorganic chlorides. For the measurement of the total organic nitrogen, sulphur and phosphorus parameters the XAD columns are washed with a solution of sodium chloride and hydrochloric acid respectively. This washing procedure is not necessary for the other analytical techniques.

Problems may occur in the Amest test when spores of bacillus are present in the water-sample. These spores remain on the XAD columns, are eluted and survive during the whole sample preparation procedure. Therefore the XAD eluates must be filtered over a 0.2 μm Teflon filter. Filtration of the water sample before the XAD adsorption has to be avoided in order to prevent loss of organic material on the filter.

Figure 4 shows the elution and filtration apparatus.

An evacuated recipient causes a constant flow of eluate through capillary C and therefore also in filter F and in the XAD column. By filling the solvent containers whilst valve *V* is closed, an airbubble in the tube between the containers will separate the two solvent types: the ethanol solvent passes the XAD column first, followed by the ethanol-cyclohexane mixture. Complete elution takes 100 minutes.

Treatment of the XAD-eluates

The eluate of an XAD-column contains water, cyclohexane and ethanol. The water and cyclohexane have to be removed and the remaining dry ethanol solution has to be concentrated to the desired volume.

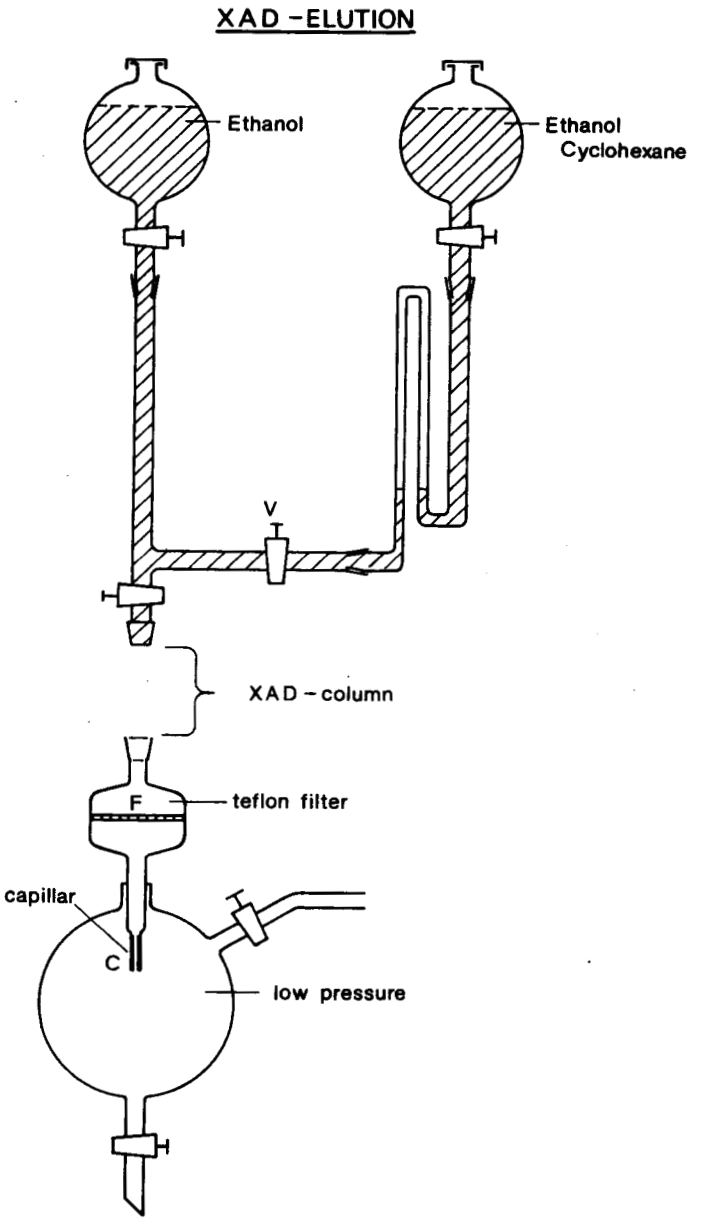

FIGURE 4 XAD-elution and filtration apparatus.

Adsorbents such as sodium sulphate and silicium oxide cannot be used to remove water as polar organic compounds will also be adsorbed. Therefore the removal of water and cyclohexane is realized in two successive azeotropic distillation steps. First all the water and part of the cyclohexane are removed in the ternary system of ethanol-cyclohexane-water (with azeotropic ratio 17:76:7) boiling at 62°C.

The remaining cyclohexane is removed in a binary ethanol-cyclohexane system (azeotropic ratio is 30.5:69.5) boiling at 64.9°C. The ethanol residue is concentrated by evaporation to a few milliliters. This working-up procedure has also been automated. As it was impossible to carry out the whole procedure in one automated programme, a two-step system was chosen.

In the first step the eluate is dried and concentrated to about 240 milliliters (see Figure 5). As soon as the volume of the eluate has reached 250 ml, a level detector will disengage the heater of the distillation apparatus, leaving about 240 milliliters of eluate.

The eluate still contains ethanol and cyclohexane. In the second step (see Figure 6) the residue of step 1 is replenished with ethanol to a volume of exactly 250 milliliters and heated in a waterbath.

After distillation of 245 ml, which can be measured by level detection in a graduated cylinder, the heated water is replaced by cold water in order to stop the distillation abruptly. With this procedure a concentration is performed from several liters of eluate to 5 milliliters of concentrate. This whole procedure can be carried out in about 10 hours.

Cleaning of the XAD-resin

It is possible to get good blank-results with all the above mentioned toxicological and chemical techniques provided that the XAD-resin and the various organic solvents have been purified to a high degree.

The XAD-resin in particular should not introduce compounds that might interfere in the Ames test or in the analytical measurements. Furthermore the resin should have an optimal adsorption capacity. The commercially available resins do not fulfil these conditions. Therefore a cleaning procedure has been developed and automated in which the resin is washed a number of times and several hot extractions are carried out.

The different cleaning-steps are:

1) Washing with diluted lye (repeated 10 times).
2) Washing with diluted hydrochloric acid (repeated 10 times).
3) Washing with distilled deionised water (repeated 10 times).
4) Washing with methanol (repeated 2 times).

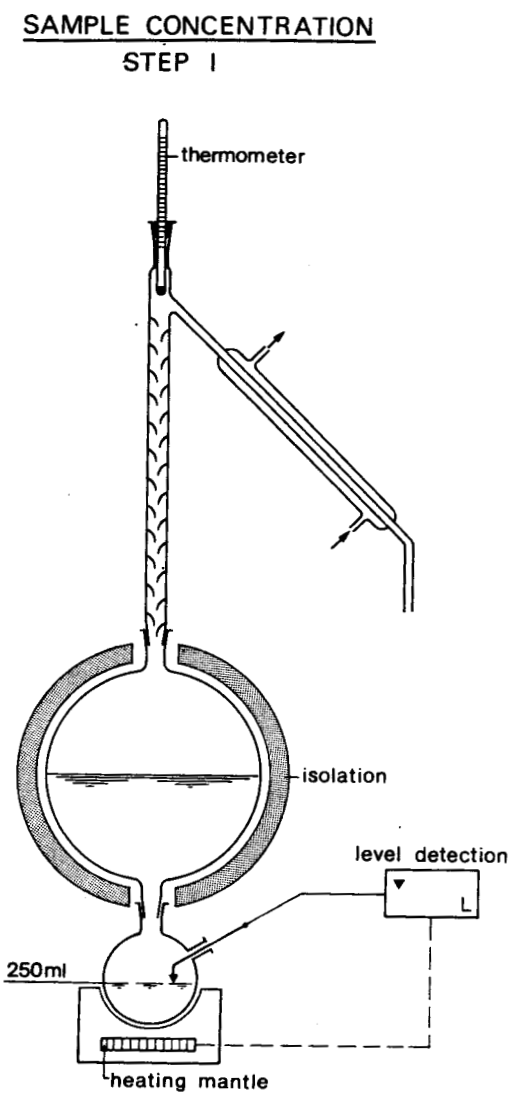

FIGURE 5 Sample concentration (step 1).

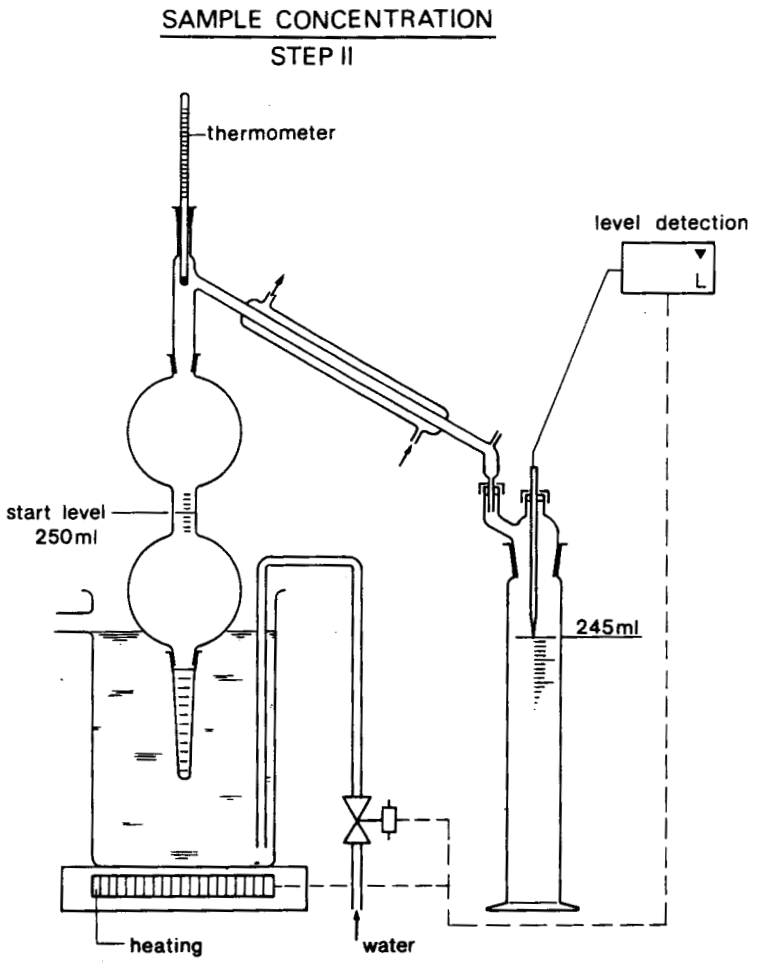

FIGURE 6 Sample concentration (step 2).

5) Soxhlet extraction with hot methanol for 24 hours.

6) Washing with ethanol (repeated 3 times).

7) Soxhlet extraction with an azeotropic mixture of ethanol and cyclohexane for 24 hours.

8) Washing with double-distilled methanol (repeated 5 times).

Figure 7 shows the automatic resin cleaning installation. The cleaning procedure shown in the table is carried out in about 90 hours in this installation.

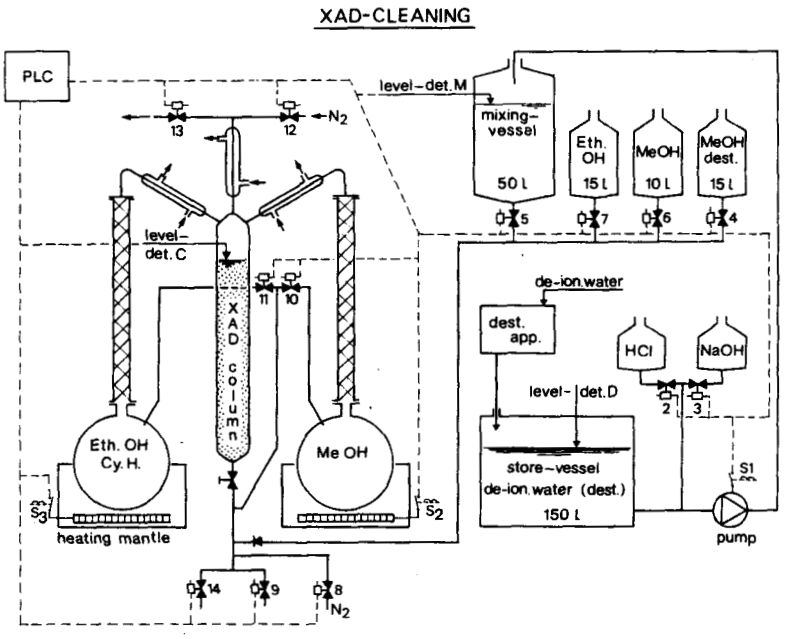

FIGURE 7 Resin cleaning installation.

The whole procedure is controlled by a P.L.C. (a Programmable Logic Controller). In each run 5 liters of resin are cleaned, which is sufficient for the isolation of 2,500 liters of water at 2 pH-values.

Personal costs

In Table II a survey is given of man hours required for the various parts of the procedure. The time mentioned refers to one water-sample at a time. If more samples are analysed at the same time the number of man hours per sample will decrease.

The isolation of organic compounds from water with XAD has proved to be a very suitable technique for an integrated toxicological and chemical determination of water quality.

The automation of the different parts of the whole procedure allows to perform this complicated technique on a routine basis for both research programmes and quality investigations in water works.

Further research has still to be done on the alkaline fraction of the isolation, the isolation of the more hydrophilic organic material and the analysis of high molecular compounds.

TABLE II

Total time consumption and real working time for a complete XAD-isolation and sample preparation procedure

	Duration	Man hours
Isolation	20 hrs	3 hrs
Elution + filtration	2 hrs	1 hr
Sample preparation	10 hrs	1.5 hr
XAD cleaning 5 l of resin for 2,500 l of water at 2 pH's	4 × 24 hrs	8 hrs

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