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Portable liquid chromatograph for mobile laboratories I. Aims

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Abstract

According to general standards existing in chemical analysis carried out in a field laboratory, the following requirements apply to field HPLC analysis: sensitivity of the method, resolution, linear dynamic range and detector sensitivity. The construction of a field liquid chromatograph includes: a column of 60–80 mm × 2 mm I.D. (sorbent with $d_p = 5$ μm; $N \approx 5000$ theoretical plates); two-syringe-type gradient pumps (2 × 2.5 ml, $P_{max} = 7$ MPa at $F = 0.005$–1 ml/min); UV spectrophotometric detector with a cell of 1.6 mm × 1 mm diameter; stop-flow injector; and column heater. The efficiency of a field chromatograph is illustrated by the examples of separations which are typical of environmental analyses in situ.

Keywords: Field laboratories; Mobile laboratories; Polynuclear aromatic hydrocarbons; Phenols; Phthalate esters; Pesticides; Explosives, polynitro; Inorganic anions

1. Introduction

During the last decade, in connection with threatening pollution to the environment, great attention has been paid to ecological investigations which, in their turn, gave the impetus to the development of analytical techniques. For example, many different universal and specialized analysers from pH meters to mass spectrometers were designed for analyses in mobile laboratories directly at the investigation sites. Chemical analysis in situ allows immediate decisions to be made and avoids expenditure connected with sample preservation and sample transport to remote laboratories.

As far as universal chromatographic equipment is concerned, at present only portable gas chromatographs are well known, e.g., Chromato-Sud (France) and Chrompack (Netherlands). Although HPLC is widely used in environmental analytical chemistry, we know of only one portable isocratic HPLC system, Minichrom II (Industrielle Mess- und Regelsysteme für Umweltechnologie, Germany) with a photometric detector (254 and 280 nm) and several portable ion chromatographs.

More than 10 years ago, we described an OB-4 liquid chromatograph (MiLiChrom) [1], the latest design of which, MiLiChrom-4, is still produced by Nauchpribor (Oryol, Russia). Over 5000 MiLiChrom chromatographs were produced. The HPLC variant proposed by us (which could be termed a "microbore short column HPLC system" according to modern terminology), appears to be very viable and, to our surprise, is well suited to solving a great number

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of problems relating to environmental analytical chemistry. Taking this fact into account, we investigated the optimization of the method and equipment, which was aimed at the creation of a portable liquid chromatograph for mobile laboratories. Later, we shall call it a "field chromatograph".

2. Experimental

Pesticides, standards of polynuclear aromatic hydrocarbons (PAHs), phenols and phthalate esters were obtained from Supelco (Bellefonte, PA, USA). Polynitro explosives were provided by A. Shishmarev (Engineering Centre, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia). Eurospher-80 5 C_{18} was supplied by Knauer (Berlin, Germany). Nucleosil 5 C_{18} PAH was kindly given by Dr. P. Wollenweber (Macherey–Nagel, Düren, Germany).

Columns were packed with a non-viscous low-concentration slurry. The field chromatograph prototype was designed and produced by the Institute of Nuclear Physics, the Institute of Supplied Microelectronics and the Novosibirsk Institute of Bioorganic Chemistry (NIBC) of Siberian Branch of the Russian Academy of Sciences, EcoNova (Novosibirsk, Russia) and our Institute. The HPLC software was written by V. Pirog and S. Dvinin (our Institute), A. Zenkov (NIBS) and Dr. Yu. Kalambet (Ampersend, Moscow, Russia).

3. Results

The first stage of resolving any optimization problem is to find the limits which will determine the areas of field liquid chromatograph application, its performance and technical characteristics. Analytical problems which can be solved are divided into three types:

(i) determination of the content of substances which can only be determined by HPLC analysis;

(ii) preliminary HPLC analysis on the basis of which information will be given on the collected samples obtained for investigations in remote laboratories;

(iii) HPLC application at the stage of sample preparation for further analysis by mass spectrometric methods.

The following are considered to be the main requirements which should be applied to a liquid chromatograph as an instrument for work in field laboratories:

(1) Minimum mass and dimensions to allow the device to be carried easily in a car, an aeroplane, a ship, etc., and for its use in small field laboratory rooms.

(2) Minimum energy consumption and efficiency with slight deviations of electric power.

(3) Efficiency over a wide range of temperature and moisture conditions, resistance to vibration, etc.

(4) Readiness to work immediately after being turned on.

(5) Minimum consumption of flammable and toxic organic solvents which demand special security measures in field laboratories.

(6) A design which allows the chromatograph to operate with equipment that is generally used in a field laboratory. The chromatograph should also use standard solutions which general field laboratory equipment requires.

(7) The chromatograph should be extremely versatile, i.e., able to solve a large number of analytical problems, and at the same time be easy to switch from one problem to another.

It is more difficult to specify technical characteristics. On the one hand, the better the characteristics, the more efficient is the method. On the other hand, it is obvious that the attempt to combine simultaneously in one device all the optimum parameters will lead to the creation of an awkward and complicated stationary chromatograph. Following the principle of "necessary sufficiency", we think that a field chromatograph should be, first of all, suitable for solving the majority of the more general problems. Their complexity is defined not only by the capability of the HPLC system, which is used in combination with other methods and procedures, but also by the level of the development of analytical chemistry.

In spite of the availability of a great variety of methods in analytical chemistry, there are a number of general regularities which are correct for HPLC. The main regularities are as follows:

(1) The molecular mass of substances defined is <500. The number of substances defined in a sample cannot exceed 15. It is more common to have only 1–5 compounds.

(2) The concentration of substance in a sample must be between 1 and 1000 mg/l. At lower concentrations, problems arise connected with losses of substance due to adsorption on vial walls, atmospheric influence, activity of microorganisms, etc. In these cases, preliminary concentration is used for the analysis.

(3) The sample volume must be at least 10 μl. It is limited by the specifications of commercially available dosing devices and by the risk of concentration changes as a result of solvent evaporation. In HPLC only 20–40% of the sample is injected into the column. As for the rest of the sample, it is used for repeated analyses or rejected. Taking this fact into consideration, the sample amount should be 2 μl. Then, the amount of substance will be 2–2000 ng for the concentration range 1–1000 mg/l.

(4) Analysis deviation should be commensurable with specifications of auxiliary equipment. If the value assumed in the range of small concentrations is 10%, then the sensitivity level for a minimum amount of the defined substance of 2 ng is 0.2 ng or 0.1 mg/l. The method deviation in the middle of the range of possible concentrations is usually not worse than 2–3%. The above limits define the linear dynamic range as 0.2–2000 ng or 0.1–1000 mg/ml.

(5) The duration of the analysis is 10–30 min. It is commensurable both with the time of sample preparation and with the time necessary to obtain a final report.

The limits mentioned above help determine the main requirements which the components of a field chromatograph must meet. Tests of certain prototypes already developed with regard to these specifications allow for the creation of a prototype field chromatograph, the scheme and specifications of which are given in Fig. 1 and Table 1. Figs. 2–10 show examples of separations

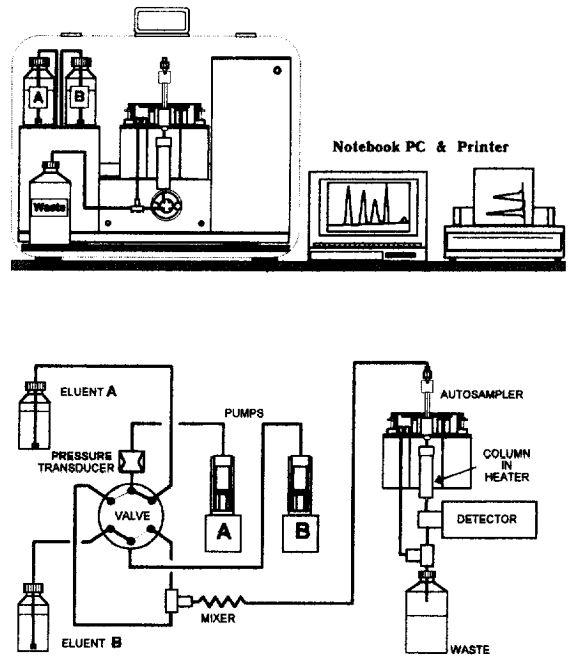

Fig. 1. Schematic diagram of the field chromatograph.

of samples of ecological toxicants typical of environmental HPLC analysis which were carried out on the prototype field chromatograph. Below, we outline the construction and technical characteristics of the components of our instrument.

3.1. Chromatographic column

The column is is the "heart" of a chromatograph, and its parameters are the starting point for optimization of other components of the instrument. In practice, all liquid chromatographs can be used with columns ranging widely in terms of size and efficiency. Such universality makes it necessary to use pumps and thermostats requiring great amounts of energy. This is inapplicable to a field chromatograph. The construction of a chromatograph with a column of one size with necessary efficiency is more appropriate.

The number of substances separated by the column depends on its efficiency N. As a rule, columns used in HPLC have an N value of not more than 10 000–20 000 theoretical plates (t.p.).

Table 1
Specifications of the field chromatograph (without computer and printer)

Component	Parameter	Specification
Columns	Diameter (mm)	2
	Length (mm)	60–80
	dp (μm)	⩾3
	Void volume (μl)	150
Detector	Double-beam multi-wavelength UV spectrophotometer [1]	
	Wavelength range in steps 2 nm (nm)	190–360
	Spectral bandwidth (nm)	10
	Wavelength accuracy (nm)	±0.5
	Wavelength reproducibility (nm)	±0.005
	Time constant and time of measurement at one wavelength, τ (s)	0.06–10
	Number of wavelengths in one cycle	Up to 8
	Noise level with cell path length 1.6 mm at $\tau = 0.3$ s and $\lambda = 260$ nm (AU)	<0.0002
	Drift with cell path length 1.6 mm at $\tau = 0.3$ s and $\lambda = 260$ nm (AU/h)	<0.0001
	Linear absorbance range with cell path length 1.6 nm (AU)	−10 to +10
	Flow-cell path length (mm); volume (μl)	1.6; 1.3
Pump	Two-syringe high-pressure gradient metering pump with switching six-port valve	
	Volume of one syringe (μl)	2500
	Flow-rate setting range (μl/min)	5–999
	Flow-rate accuracy at 100 μl/min (%)	±1
	Flow-rate reproducibility at 100 μl/min (%)	±0.3
	Maximum pressure (MPa)	7
Column heater	Solid type	
	Temperature (ambient temperature + 10°C) control range in 1°C steps (°C)	35–90
	Temperature control precision (°C)	±0.1
Autosampler	Stop-flow sample autoinjection programmed method	
	Number of vials; number of injections; volume of vial (μl)	46:50:200
	Sample volume setting range in 1-μl steps (μl)	1–100
	Injection reproducibility with 2-μl sample (%)	±0.5
Dimensions and mass		Width 530 × diameter 200 × height 300 mm; 14 kg
Power requirements		100–120/200–240 V; 100 V A; 50–60 Hz

However, many workers believe that a column with $N = 3000–5000$ t.p. is suitable for the solution of many problems [2–7]. Using different approaches of interdependence of parameters such as column length, particle dimension of sorbent, flow-rate of eluent, pressure of column inlet and time of separation, it appears that this column is optimum for routine HPLC analysis.

The separation power of the column may be characterized by the value of its peak capacity Z, equal to the maximum number of peaks with the same area which can be plotted on a chromatogram at given values of capacity factors of the first and last peaks, k'_1 and k'_m, and at fixed resolution of two neighbouring peaks, R_s with isocratic elution [8]:

$$Z = \sqrt{N}[\ln(1 + k'_m) - \ln(1 + k'_1)]/4R_s \quad (1)$$

Hence, 36 substances ($Z = 36$) can be separated

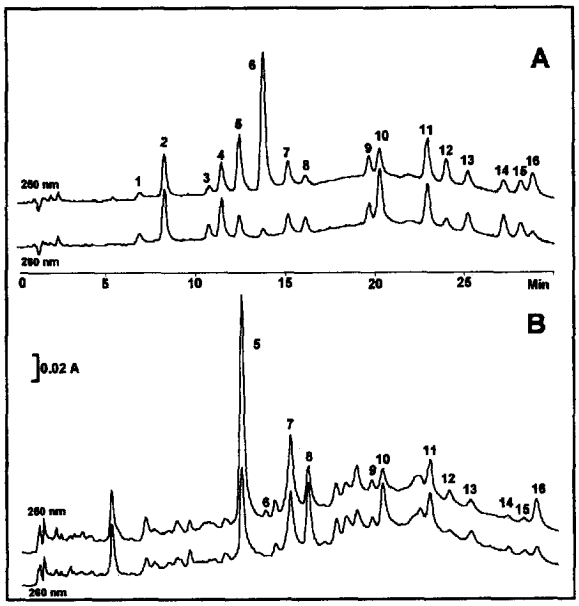

Fig. 2. Separation of sixteen polynuclear aromatic hydrocarbons. Column, Nucleosil 5-C_{18} PAH (75 mm × 2 mm I.D.). Eluents: A = methanol–water (65:35); B = acetonitrile–water (85:15). Gradient: 100% A for 3 min, 0–100% B in 30 min, 100% B for 3 min. $F = 0.12$ ml/min; $\lambda_1 = 250$ nm; $\lambda_2 = 260$ nm; $P = 1.5$ MPa; $T = 40$°C. (A) Sample 5 μl of standard methanolic solution. Peaks: 1 = naphthalene (21 ng); 2 = acenaphthylene (9 ng); 3 = acenaphthene (43 ng); 4 = fluorene (8 ng); 5 = phenanthrene (4 ng); 6 = anthracene (2 ng); 7 = fluoranthene (10 ng); 8 = pyrene (4 ng); 9 = benz[*a*]anthracene (7 ng), 10 = chrysene (7 ng); 11 = benzo[*b*]fluoranthene (9 ng); 12 = benzo[*k*]fluoranthene (5 ng); 13 = benzo[*a*]pyrene (5 ng); 14 = dibenzo[*a,h*]anthracene (15 ng); 15 = benzo[*ghi*]perylene (15 ng); 16 = indeno[1,2,3-*cd*]pyrene (11 ng). (B) Sample 2 μl of methanolic solution of hexane extract of snow water. The following concentrations of PAHs were founded in water: 5, 1.9; 6, 0.1; 7, 1.1; 8, 1.0; 9, 0.1; 10, 0.2; 11, 0.3; 12, 0.2; 13, 0.2; 14, 2.1; 15, 2.0; 16, 0.4 ppb.

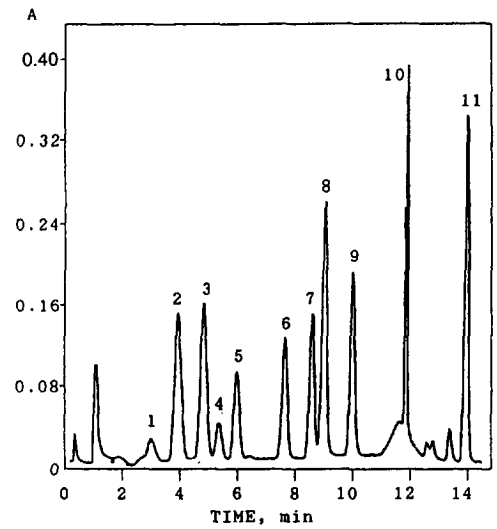

Fig. 3. Separation of eleven phenols. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluents: A = methanol–water–trifluoroacetic acid (36:63.9:0.1); (B) = methanol. Gradient: 0–68% B in 11 min, to 100% B in 1 min, 100% B for 5 min. $F = 0.15$ ml/min; $\lambda = 230$ nm; $P = 4$ MPa; $T = 50$°C. Sample: 5 μl (225 ng each of component). Peaks: 1 = phenol; 2 = 4-nitrophenol; 3 = 2,4-dinitrophenol; 4 = 2-nitrophenol; 5 = 2-chlorophenol; 6 = 2-methyl-4,6-dinitrophenol; 7 = 2,4-dimethylphenol; 8 = 4-chloro-3-methylphenol; 9 = 2,4-dichlorophenol; 10 = 2,4,6-trichlorophenol; 11 = pentachlorophenol.

on a column with $N = 5000$ t.p. at $k'_1 = 0$, $k'_{36} = 5.34$ and $R_s = 1.2$ (the overlap of neighbouring peaks is not more than 0.5%). Chromatograms obtained on the computer are given in Fig. 11.

The column length L necessary for obtaining $N = 5000$ t.p. is defined by its packing quality, i.e., by the height equivalent to a theoretical plate (HETP or H). The most popular stationary phases in HPLC are sorbents with particle size $d_p = 5$ μm. This is due to many reasons [2–7]. For commercially available columns, $H < 2.5d_p$ (or $H < 12.5$ μm). From the equation $L = NH$ it follows that $L \approx 65$ mm for a column with $N = 5000$ t.p.

As a parameter, the column volume, V_c, is seldom mentioned in HPLC, although this parameter characterizes chromatographic systems. The diameter of the column plays an important role in the classification of various HPLC systems, e.g., conventional HPLC, HPLC on narrow columns and capillary HPLC. Nevertheless, V_c is an important parameter. Equations including V_c as a dead volume V_0 are fundamental:

$$V_r = V_0(1 + k') \quad (2)$$

$$N = (V_r/\sigma)^2 \quad (3)$$

where V_r = retention volume of compound and σ = standard deviation of a Gaussian peak.

The first variable which determines V_c is the maximum admissible amount of a substance which can be injected on to a column without

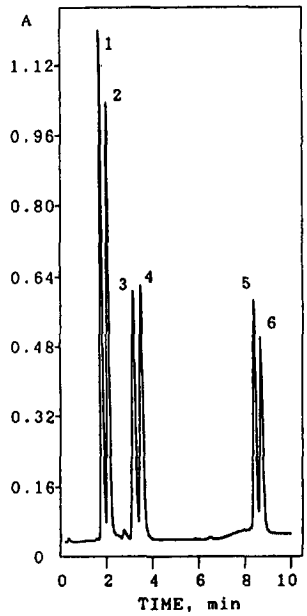

Fig. 4. Separation of phthalate esters. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluents: A = acetonitrile–water (80:20); B = acetonitrile. Gradient: 100% A for 3 min; 0–100% B in 1 min; 100% B for 4 min. $F = 0.1$ ml/min; $\lambda = 230$ nm; $P = 2$ MPa; $T = 50$°C. Sample: 2 μl (400 ng each of component). Peaks: 1 = dimethyl phthalate; 2 = diethyl phthalate; 3 = di-*n*-butyl phthalate; 4 = butyl benzyl phthalate; 5 = bis(2-ethylhexyl) phthalate; 6 = di-*n*-octyl phthalate.

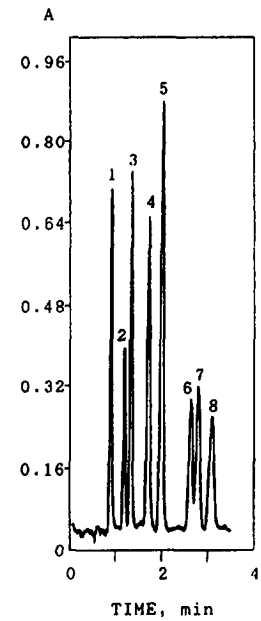

Fig. 5. Separation of polynitro explosives. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluent: methanol–water–0.1 *M* tetrabutylammonium phosphate (pH 6.8) (50:40:10). $F = 0.28$ ml/min; $\lambda = 230$ nm; $P = 5$ MPa; $T = 45$°C. Sample: 3 μl. Peaks: 1 = HMX (150 ng); 2 = RDX (150 ng); 3 = TNB (75 ng); 4 = tetryl (150 ng); 5 = TNT (75 ng); 6 = 2,4-DNT (150 ng); 7 = 2,6-DNT (150 ng); 8 = picric acid (150 ng).

overloading. Done [9] showed that typical silica-based normal and reversed phases (pores of 5–15 nm, surface area 200–400 m^2/g) have loads of 10 μg/g for low-molecular mass substances. Assuming that 1 g of such a sorbent occupies a volume of 1 ml, then it is easy to determine that close to the upper limits of the linear dynamic range of the chromatograph, $V_c = 0.2$ ml and $V_0 \approx 0.15$ ml when the sample amount is at its maximum capacity, 2 μl. For a column with $L \approx 65$ mm and with $V_c \approx 0.2$ ml, the diameter is $D \approx 2$ mm.

3.2. Detector

It is advisable to use a UV spectrophotometer as a detector for a field chromatograph, which is more common in HPLC. Following the appearance of indirect photometric detection, it became universal, i.e., it is suited for the determination of all substances including non-UV-absorbed compounds [10]. The sensitivity of a photometric detector corresponding to the requirements given above is defined for substances with a molar absorption coefficient ε_M according to Beer's law:

$$A = \varepsilon_M C_M l \tag{4}$$

where A = absorption of substance solution, C_M = molar concentration of substance solution and l = optical path length of sample cell, and according to equations for peak height of Gaussian shape with area S (S and h are proportional to the substance quantity) [11]:

$$h = \frac{S}{\sigma\sqrt{2\pi}} \tag{5}$$

It follows from Eqs. 2, 3 and 5 that

$$h = \frac{S\sqrt{N}}{V_0(1+k')\sqrt{2\pi}} \tag{6}$$

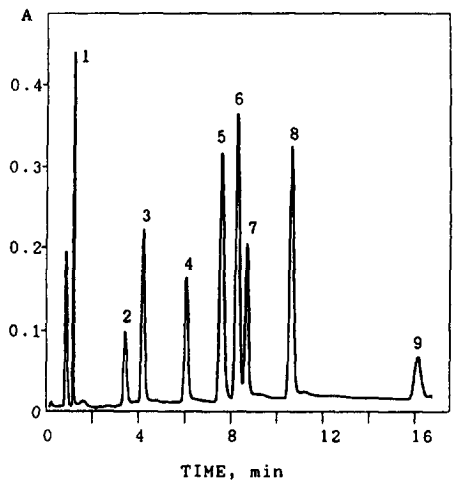

Fig. 6. Separation of triazine and similar pesticides. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluents: A = acetonitrile–water–1 *M* sodium acetate (pH 5.75) (30:69:1); B = acetonitrile–water–1 *M* sodium acetate (pH 5.75) (50:49:1). Gradient: 0–100% B in 10 min; 100% B for 6 min. $F = 0.2$ ml/min; $\lambda = 220$ nm; $P = 3$ MPa; $T = 45$°C. Sample: 2 μl. Peaks: 1 = bentazon (100 ng); 2 = tebuthiuron (200 ng); 3 = simazine (100 ng); 4 = atrazine (100 ng); 5 = prometon (100 ng); 6 = propazine (100 ng); 7 = guthion (100 ng); 8 = prometryn (100 ng); 9 = diazinon (400 ng).

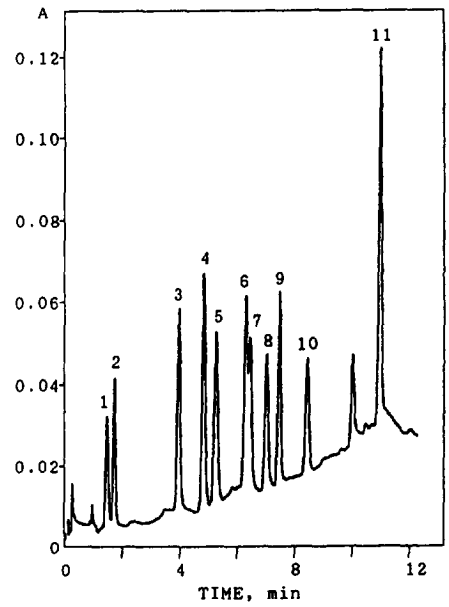

Fig. 7. Separation of carbamate and urea pesticides. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluents: A = methanol–water–1 *M* sodium acetate (pH 5.0) (35:64:1); B = methanol–water–1 *M* sodium acetate (pH 5.0) (85:14:1). Gradient: 0–100% B in 10 min; 100% B for 3 min. $F = 0.2$ ml/min; $\lambda = 240$ nm; $P = 4$ MPa; $T = 45$°C. Sample: 2 μl. Peaks: 1 = bentazon (20 ng); 2 = methomyl (20 ng); 3 = aldicarb (200 ng); 4 = bendiocarb (200 ng); 5 = tebuthiuron (40 ng); 6 = propachlor (200 ng); 7 = diuron (20 ng); 8 = propanil (20 ng); 9 = chlorpropham (20 ng); 10 = metolachlor (200 ng); 11 = triallat (200 ng).

When the volume of the injection sample to a column is V_s, its absorbance is A_s and its concentration is C_s, the peak area can be expressed as follows:

$$S = A_s V_s = \varepsilon_M C_M l V_s \tag{7}$$

Then, Eq. 6 becomes

$$A_p = \frac{\varepsilon_M C_s l V_s \sqrt{N}}{V_0(1+k')\sqrt{2\pi}} \tag{8}$$

where A_p = peak height in absorbance units (AU).

Assuming that a typical substance has $\varepsilon_M = 10^4$ mol^{-1} *l* cm^{-1} and $k' = 2$, then *h* can be calculated for a column with $V_0 = 0.15 \cdot 10^3$ l and $N = 5000$ t.p. having limiting conditions $C_s = 10^{-6}$ *M*, $V_s = 2 \cdot 10^{-6}$ l. If $l = 1$ cm, then $A_{min} \approx 0.001$ AU. The value obtained will be the limit of the detector sensitivity. The value of A_{min}, which corresponds to the sensitivity limit, determines the detector noise level. In chromatography, the limit of detector sensitivity is determined by the amount of the substance, the peak of which on a chromatogram is equal to amplitude of double noise A_n [12]. According to the equation

$$A_n = 0.5 A_{min} \tag{9}$$

it follows that A_n should not exceed 0.0005 AU.

The upper limit of the detector range can be determined from Eq. 8. Under the same conditions, the upper concentration limit of the method is $A_{max} = 12.5$ AU for a sample with concentration $C = 10^{-2}$ *M*. It is impossible to measure such a large absorption in a cell of $l = 1$ cm. Usually, the necessary linearity (deviation not more than 1%) reached in photometers is up to a value $A \approx 2$ AU. According to Eq. 4, the value of *l* should be six times less, i.e., 1.66 mm.

The volume of the sample cell is defined as the

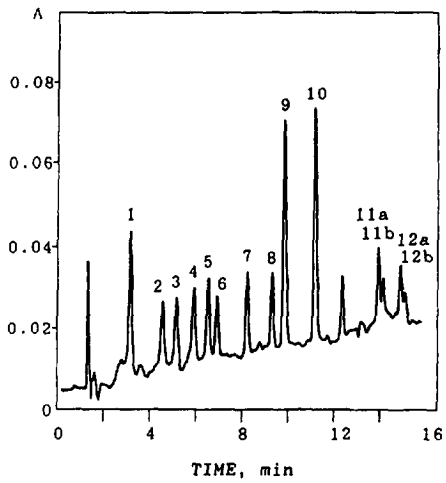

Fig. 8. Separation of pesticides: chlorinated aromatic acids and esters. Column: Eurospher 80-5 C_{18} (64 mm × 2 mm I.D.). Eluents: A = methanol–water–acetic acid (100:100:1); B = methanol–water–acetic acid (180:20:1). Gradient: 0–20% B in 1 min; 20–100% B in 12 min; 100% B for 3 min. $F = 0.15$ ml/min; $\lambda = 220$ nm; $P = 3$ MPa; $T = 45$°C. Sample: 2 μl. Peaks: 1 = Banvel-D (620 ng); 2 = 2,4-D acid (320 ng); 3 = diuron (320 ng); 4 = 2,4,5-T acid (320 ng); 5 = 2,4-D methyl ester (320 ng); 6 = 2,4-DB acid (320 ng); 7 = 2,4,5-T methyl ester (320 ng); 8 = 2,4-DB methyl ester (320 ng); 9 = dacthal (320 ng); 10 = trifluralin (internal standard) (80 ng); 11a, 11b = 2,4-D (isooctyl ester) (620 ng); 12a, 12b = 2,4,5-T (isooctyl ester) (620 ng).

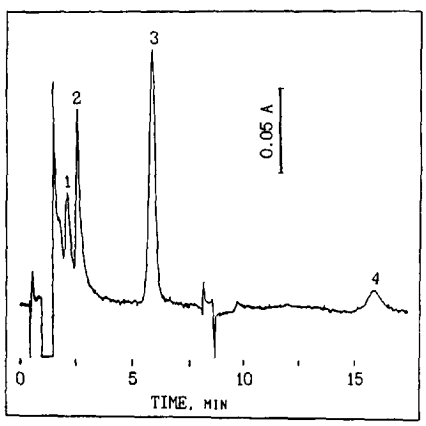

Fig. 9. Determination of the main inorganic anions in snow water with indirect photometric detection. Column: Nucleosil 5-C_{18} (64 mm × 2 mm I.D.) dynamically coated with tridecylbenzylammonium bromide. Eluent: 1.6 mM potassium phthalate (pH 6.0) in acetonitrile–water (1:9). $F = 0.2$ ml/min; $\lambda = 240$ nm; $P = 3$ MPa; $T = 25$°C. Sample: 40 μl of snow water. Peaks: 1 = $H_2PO_4^{2-}$ (3.2 ppm); 2 = Cl^- (1.1 ppm); 3 = SO_4^{2-} (8.3 ppm); 4 = NO_3^- (1.1 ppm).

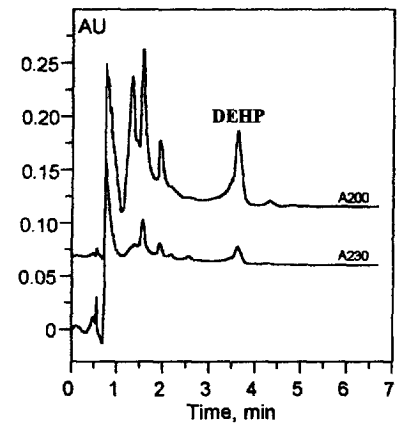

Fig. 10. Direct determination of bis(2-ethylhexyl) phthalate (DEHP) in water from Lake Baikal (depth 900 m; August 1995). Column: Nucleosil 5-C_{18} (75 mm × 2 mm I.D.). Eluent: methanol–water (90:10) (pump B). $F = 0.3$ ml/min; $\lambda_1 = 200$ nm; $\lambda_2 = 230$ nm; $P = 4$ MPa; $T = 35$°C. Sample: 6000 μl of sampled water–2-propanol (95:5) pumped with pump A at 300 μl/min. The concentration of DEHP was found to be 1.7 μg/l.

acceptable value of the extra-column dispersion of the substance zone. This value also includes the zone dispersion of the injector and connecting tubes. These values are connected by the equation

$$\sigma^2_{peak} = \sigma^2_{col} + \sigma^2_{ext} \quad (10)$$

where σ_{peak}, σ_{col} and σ_{ext} are the standard deviations of chromatographic peak, column and extra-column contribution, respectively. It is accepted in HPLC [12] that

$$\sigma^2_{ext} < 0.1\sigma^2_{col} \quad (11)$$

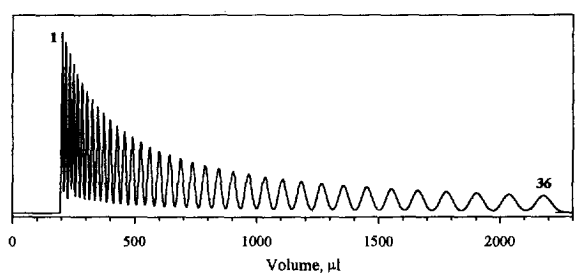

Fig. 11. Computer-simulated chromatograms as illustrations of peak capacity of short column according Eq. 1. $N = 5000$ t.p.; $V_0 = 0.165$ ml; $k'_1 = 0$; $k'_m = 5.34$; $R_s = 1.2$; $Z = 36$.

Assuming to a first approximation that

$$\sigma^2_{cell} \approx \sigma^2_{ext} \approx 0.1\sigma^2_{col} \quad (12)$$

it is possible to estimate the value of the maximum admissible zone of dispersion in the cell detector. According to Eqs. 2, 3 and 12, it follows that

$$\sigma_{cell} \approx 0.32V_0(1 + k')/\sqrt{N} \quad (13)$$

Therefore, during the elution of an unretained compound (the narrowest peak, $k' = 0$), σ_{cell} should not exceed 0.68 μl on a column with $N = 5000$ t.p. and $V_0 = 150$ μl.

It is difficult to identify precisely the volume of the cell detector, because it is defined by its form. It is easier to find the form and the volume of the cell experimentally with regard to the necessary value of σ_{cell}. Earlier we showed that a cell of the Z-type form with diameter 1 mm, $l = 1.6$ mm and $V_{cell} = 1.26$ μl possesses good characteristics [1]. The dispersion of a very narrow zone of the solution ($V = 0.1$ μl) of a UV-absorbed substance which is injected into the cell through a capillary of 50 mm × 0.15 mm I.D. is characterized by the value $\sigma \approx 0.6$ μl having a methanol flow-rate of 100 μl/min through the cell.

3.3. Injector

With virtually all liquid chromatographs, sample injection on to the column is carried out with the help of a loop-type injector without stopping the column flow. Nevertheless, we think that for a field chromatograph, a more suitable method for sample injection is to stop the column flow by means of a chromatographic syringe-type pump. As was shown previously [1], it has a good repeatability, is universal with regard to sample amount and operates with fewer high-pressure connections. The efficiency of a column with such an injector is high. The step motor of the pump [1] with 200 steps per 360° provides a dosing accuracy of 0.05 μl per step, i.e., ±0.5% of a 2-μl sample amount.

With regard to the advantages of stop-flow injection, we are concerned only with chromatographs with photometers that are hardly sensitive to the stop-flow injector.

3.4. Pump

Requirements for the pump are mainly determined by the size of the column and the particle size of the sorbent, and by the type of detector. They include such characteristics as the range of flow-rates, maximum pressure and stability of flow.

According to theoretical suppositions and a great deal of known experimental data, it follows that the optimum linear flow-rate of the eluent through the column with a sorbent of $d_p = 5$ μm is about 1 mm/s. Maximum efficiency is achieved at such a flow-rate. For a column with $D = 2$ mm, the optimum volume flow-rate of the eluent is $F_{opt} \approx 200$ μl/min. The necessary column inlet pressure P_{opt} (MPa) can be calculated from the equation [6]

$$P = 21.7F\eta L/(Dd_p)^2 \quad (14)$$

where F = flow rate (ml/min), η = viscosity of mobile phase (cP), L = length of column (mm), D = column diameter (mm) and d_p = diameter of stationary phase particles (μm). For aqueous organic eluents ($\eta = 1.5$–2 cP at 25°C), P will be 4–6 MPa.

One of the important characteristics of the pump is the inconstancy of flow. The retention of a compound is directly connected with the value F. In order to achieve repeatability of retention, the flow pulsation should not be higher than 1%.

It is very easy to achieve such characteristics by means of a syringe-type pump. Reciprocating piston pumps of small volumes noticeably pulsate, and they are used with a special pulse damper. As was mentioned before, syringe pumps can be used for sample injection into the column. A detailed description of the characteristics of a syringe-type pump was given by Martin et al. [13]. The main peculiarity is connected with the compressibility of liquid in the syringe. As a result, when the separation starts from $P = 0$, non-correspondence of flow-rates of the eluent and of the linear movement of plunger

is observed during the initial stage of elution. These rates correspond again on reaching a stationary pressure. This leads to an increase in experimentally determined retention times and peak areas with regard to true values. In order to minimize errors due to the pump, it is necessary to reduce the volume occupied by the eluent between the pump plunger and the column to the volume necessary for the performance of only one run.

In our case, the volume is 5600 μl. It consists of the volumes of the two syringes (2500 μl each) and of the total volume of the connecting capillaries, mixer and valve (600 μl). Such a volume of eluent is sufficient for column regeneration with dead volume $V_0 = 150$ μl and $N = 5000$ t.p. and for subsequent isocratic and gradient elution without refilling the pump syringes.

The reduction of the liquid volume which occupies V_p at pressure P can be calculated from the equation:

$$\Delta V = V_p(1 - e^{-\chi P}) \quad (15)$$

where χ is the coefficient of liquid compressibility [14]. For $V_p = 5600$ μl of methanol ($\chi = 1.25 \cdot 10^{-3}$ MPa at 25°C) and $P = 5$ MPa, $\Delta V = 34$ μl, which is five times less than the retention volume of the unretained compound (V_0).

It should be taken into account that owing to liquid compressibility, the flow-rate of eluent through the column F (ml/min) will achieve the displacement speed of the plunger Q (cm^3/min) only at infinite time. The time necessary for achieving a flow-rate close to the defined value can be calculated as

$$t = \frac{V_p}{Q}\left[1 - \left(1 - \frac{P}{P_\infty}\right)^{\chi P_\infty}\right] \quad (16)$$

where P = achieved pressure and P_∞ = pressure drop at infinite time [13]. This is the term necessary for HPLC: $F = 99\%$ Q. If $Q = 200$ μl/min and $P_\infty = 5.00$ MPa, then $F = 198$ μl/min at $P = 4.95$ MPa, as P is proportional to F. As follows from Eq. 16, P will achieve 4.95 MPa for $V = 5600$ μl filled with methanol in $t \approx 0.8$ min. During this period, the plunger will move 160 mm^3 forwards, and the volume of eluent passing through the column will be $\Delta V = 34$ μl less, i.e., 126 μl. The value obtained is less than $V_0 = 150$ μl. This ensures the elution of even unretained compounds at a constant flow-rate.

Temperature (T) has an important role in HPLC, for the following reasons:

(1) As T increases, the eluent viscosity decreases and, as a result, the pressure drops. The maximum T in HPLC does not exceed 70–80°C, since the majority of eluents used do not have high boiling points. When columns are often used at $T > 55$–60°C, their lifetime is reduced significantly.

(2) T is an important parameter which influences selectivity. Variations in T are used to optimize the separation [14,15].

(3) The control of column T is necessary for the repeatability of the retention time. In typical cases, the dependence of $\ln k'$ on $10^3/T$ (K) is linear in the range 10–60°C and more sensitive to temperature changes of substances having a large k' [14,15]. As the retention is proportional to k', it is obvious that the maximum error in the retention determination will be mainly for such substances. The sensitivity of $k' \approx 10$ to temperature changes is about 1 at 10°C [14]. Therefore, the value of the deviation acceptable in HPLC for the retention determination, which equals 1%, could be achieved with an accuracy of temperature control of the column better than 1°C.

As a field chromatograph is designed to work with columns of only one size, the most suitable is a solid-type column heater which differs from air ovens by being more compact, requiring less electrical power and of simpler, more reliable construction.

3.5. Field chromatograph

The result of our discussions is the scheme of a chromatograph which corresponds to requirements given above and designed for HPLC analysis under field conditions (Fig. 1). Two-syringe-type gradient pumps consist of a fill–empty valve, a pressure transducer and a static mixer. The pump is connected with the column by means of a needle. The latter, together with a

tightening device, comprises an injector of the stop-flow type. Pump A collects samples into the needle. The column is placed in a solid-type column heater. The compact autosampler (46 vials) guarantees precision of the injection procedure (R.S.D. < 1% from 2 to 100 μl). The detector is a double-beam UV spectrophotometer with a special drive for rapid changes of wavelengths according to stepwise cyclic programmes (up to 8λ per cycle) over the range 190–360 nm [1]. The chromatograph is operated by a notebook personal computer. The arrangement principles of the syringe-type pump, the injector, the column and the detector were described earlier [1]. However, some of their technical characteristics have been improved (see Table 1).

The next paper will be devoted to a detailed description of the construction of such a chromatograph. In this paper we should mention that the mass of the prototype chromatograph does not exceed 15 kg and the dimensions are not more than width 530 × diameter 200 × height 300 mm. Figs. 2–10 show chromatograms which illustrate the capabilities of the chromatograph with gradient and isocratic elution. They illustrate the typical sensitivity for HPLC analysis. All the separations discussed in the previous paper [1] could be added to them, as they were carried out on the same type of equipment.

4. Discussion

Having formulated all of the requirements for a field liquid chromatograph, we established the main technical requirements of its components by simple calculations, and on that basis the prototype was designed. This chromatograph does not correspond to the main trends in HPLC development in many respects, and we would like to discuss this problem in detail.

Concerning the modern state of HPLC analysis, we took into account all typical analytical problems. Our conclusions are confirmed to a large extent by wide statistical data based on a questionnaire given to many specialists in the field of HPLC and on reviews of these data [16–18]. It follows that:

(i) the concentration of a "ready for injection" sample is >1 mg/l in 70% of cases;

(ii) more than 60% of the samples which are "ready for injection" have a volume of 1–10 ml; as the sample volume injected on to a column rarely exceeds 10 μl, its usage coefficient is small, and it can and should be increased;

(iii) the deviation value of the analytical procedure in 55% of cases is 1.6–5.0%.

We did not find statistical information concerning simultaneously determined components in one sample. According to our data, obtained from statistical treatment of 199 papers published in 1985 and 1986 in *Chromatographia* and *Analytical Biochemistry*, in half of those papers there were data on the simultaneous analysis of 1–5 components, in 85% less than ten substances and only in 15% more than ten components in one sample; 80% of them involved analyses of amino acids and peptides. However, this does not correspond to the analysis of environmental samples.

A special aspect of HPLC is the problem of column efficiency. Asserting that for a field chromatograph a column efficiency of 5000 t.p. is adequate, we understand that this estimation is suspect, and could hardly be justified. If only one optimum criterion is chosen, such as separation power, it should be very difficult to demonstrate the superiority of one system of HPLC analysis over another. HPLC is not just a separation, and especially it is not "separation at any cost". Configuration of this fact could be found in the opinions of various workers [2–6]. This confirmation is in favour of columns with $N \approx 5000$ t.p., or in statistics. At first, the latter does not seem favourable for our work. Several studies [16–18] indicate that the most popular column in analytical HPLC in 1991–94 had dimensions 200–250 mm × 4.6 mm I.D. Such a "mean statistical" column has $N = 15\,000$–$20\,000$ t.p. with a sorbent of the C_{18} type having $d_p = 5$ μm.

These data coincide with ours which correspond to 1985–86. However, we obtained an unexpected result when we reviewed 179 published chromatograms and estimated the real column efficiency: in 34% of the chromatograms

it is 1000–3000 t.p., in 42% it is 3000–6000 t.p. and in only 6% it did exceed 12 000 t.p. Moreover, in more than 50% of the cases, the columns have excessive efficiency, as they provide a resolution $R_s > 2$. These data are 6 years old, but the picture has not changed.

There are three answers, at least, to the question, "Why is HPLC on short columns not widespread?":

(1) One of the reasons is connected with pumps for HPLC. Beginning from the 1970s, the main pumps in HPLC were reciprocating piston pumps. More than 80% of chromatographs are equipped with such pumps [19]. At pressure drops lower than 5 MPa necessary for short columns, these pumps produce a noticeable pulsating flow, and are not suitable for work with highly sensitive detectors.

(2) Detectors used for work with common columns have flow cells with $V \approx 10$ μl. Although there are complete sets of microcells, they demonstrate lower characteristics.

(3) Columns of small volume are poorly compatible with routine chromatographic equipment, such as the injector, thermostat and autosampler. Problems connected with extra-column band broadening do not allow for the achievement of their full efficiency.

Hence, the large market for standard chromatographic equipment became a factor which restrained the development of HPLC in the direction of the production of short columns. Although such columns are still produced, they are packed with adsorbents in which $d_p = 3$ μm and are for "fast" chromatography when the pressure is not less than 8 MPa.

On the other hand, there is a sphere of HPLC analysis where columns of very small volume are still used. It started 40 years ago [20,21]. The results obtained on columns with volumes ranging from 50 to 200 μl with "soft" stationary adsorbents were good for that time [22]. The application of the same instruments and columns with "hard" adsorbents brought about the introduction of microscale HPLC or capillary HPLC [23].

Although many years have passed, this method is still not used in routine analytical practice; it is a version for enthusiasts. The proportion of columns used with I.D. < 1.5 mm in 2%, and it has remained practically the same since 1988 [16–18]. That is why the production of Famillic-100 chromatographs (Jasco) and some others for work with capillary columns was stopped. Only ISCO continues to advertise equipment (e.g., LC-500 pump, LC-10 detector and columns of 100–250 mm × 1 mm I.D. with 3-μm adsorbents) which can be also used in supercritical fluid chromatography. Having the advantages of increased sensitivity and economical use of solvents, the low popularity of capillary HPLC is connected with its non-correspondence to other methods of analytical chemistry. Standard laboratory equipment, with its limited scale of manipulation, is unable to operate with capillary HPLC equipment. These are the main technological reasons which obstruct the use capillary HPLC in environmental analysis in the field.

Hence there is a gap between scales of conventional and capillary HPLC which corresponds to the requirements of field HPLC. In order to fill this gap, it is necessary to develop special chromatographic equipment, as it is impossible to use the old equipment any longer. If one is to develop a chromatograph with only a 50–100 mm × 2 mm I.D. column, that chromatograph can be optimized, and its design will be very simple and reliable, which is not applicable to the universal chromatograph with a 50–200 mm × 1–4 mm I.D. column.

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