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Large injection volumes in capillary liquid chromatography: Study of the effect of focusing on chromatographic performance

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ABSTRACT

This paper describes a multivariate approach to study the effect on chromatographic conditions and to optimize such conditions in capillary liquid chromatography when high injection volumes are required. Several separations have been evaluated by using isocratic and gradient solvent elution, as well as isocratic elution combined with temperature programming. In this study, easily ionisable organic compounds with low log *P* have been used as representative analytes. Injection volume and nature of the injection solution have been evaluated in order to increase the sensitivity (peak area) and column performance (*N* values). The equations obtained by multiple linear regressions and response surfaces allow achieving the optimum on-column focusing conditions for chlorophenoxy acids, carbamates and heterocyclic amines.

1. Introduction

The most common method for the determination of traces and ultra traces of ionisable or thermally unstable organic compounds is liquid chromatography (LC) with different detectors in combination with pre-treatment methods. The determination of organic pollutants in soils, water or food has been described using a wide variety of techniques to preconcentrate and purify the analytes, such as liquid–liquid extraction, solid phase extraction (SPE), on-line SPE and solid phase microextraction.

Capillary LC (cLC) with packed 0.1–0.5 mm inner diameter columns has been established as an alternative technique to conventional sized LC [1]; cLC is environmentally friendly (low consumption of solvents, samples and reagents, and low waste), cost saving and, in general, better suited for screening and/or for coupling separation techniques to each other and with various detection techniques. In addition, the relatively low heat capacity and the small diameter of the columns allow using temperature programming [2,3]. Opposite to other miniaturized techniques like capillary electrophoresis, up- and down-scaling of capillary LC methods is possible by adjusting the separation to the actual analytical requirements, e.g., with respect to the available sample amount [1]. A general problem in cLC is the loss of sensitivity due to the small volumes or masses injected. This problem is associated with the need to adapt injection volume to the size of the column to prevent band broadening. In some cases, this problem can be overcome by the use of so-called on-column focusing techniques with large injection volumes [1–6]. In these techniques, the sample solvent has significantly lower elution strength compared to that of the mobile phase at the beginning of the chromatographic run [7–9] or is set at a lower temperature than that of the mobile phase [2]. For analytical separations, it is usually preferable that the sample is dissolved in the mobile phase. In this case, there is no difference in solvent strength (*k* values) between the sample solvent and the mobile phase. Regarding large sample volumes, they could be used when resolution is not a limiting factor or when the sample is dissolved in a solvent with elution strength lower than that of the mobile phase [10].

Two strategies could be used when preconcentration is needed in trace analysis before separation in cLC or micro liquid chromatography (microLC): either solid phase extraction or solid phase microextraction alone, or any of those combined with large volume injections of the sample. Extraction and preconcentration of polar and acidic organic compounds at trace levels have been carried out by employing several methods, mainly solid phase extraction (SPE), on-line SPE [5,7,11–17] and solid phase microextraction (SPME) [13]. All these preconcentration methods are even more critical in cLC or microLC that in conventional HPLC, due to the low elution strength required for focusing sample solutions, because of the high solubility of polar and acidic compounds in water. Therefore, preconcentration step and cLC determination with high injection volumes must be made compatible by using focusing method-

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Table 1
Characteristics of the analytes included in the study.

Compound	pK_a	log P
2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx, CAS no: 77500-04-0)	5.95	1.08
9H-Pyrido[3,4-b]indole (norharman, CAS no: 244-63-3)	6.80	2.80
1-Methyl-9H-pyrido[3,4-b]indole (harman, CAS no: 486-84-0)	7.16	2.71
7-Methoxy-1-methyl-9H-pyrido[3,4-b]indole (harmine, CAS no: 442-51-3)	7.70	3.22
2,4-Dichlorophenoxyacetic acid (2,4-D, CAS no: 94-75-7)	2.73	2.80
2-methyl-4-chlorophenoxyacetic acid (MCPA, CAS no: 94-74-6)	3.05	1.77
2,4-Dichlorophenoxyacetic methyl ester (2,4-D-1-methyl ester, CAS no: 1928-38-7)	–	2.90
4-(2,4-Dichlorophenoxy)butyric acid (2,4-DB, CAS no: 94-82-6)	4.80	3.53
2,4-Dichlorophenoxyacetic butyl ester (2,4-D-1-butyl ester, CAS no: 94-80-4)	–	4.40
2-(2,4,5-Trichlorophenoxy)-propanoic acid (2,4,5-TP, CAS no: 93-72-1)	3.60	3.80
2-(2,4-Dichlorophenoxy)-propanoic acid (2,4-DP, CAS no: 120-36-5)	3.00	3.43
2-(4-Chloro-2-methyl)-phenoxypropanoic acid (MCPP, CAS no: 96-65-2)	3.75	3.13
4-(4-Chlroro-2-methylphenoxy)-butanoic acid (MCPB, CAS no: 94-81-5)	3.10	3.50
1-Naphthyl methylcarbamate (carbaryl, CAS no: 63-25-2)	–	2.34
4-(Dimethylamino)-3-methylphenyl methylcarbamate (aminocarb, CAS no: 2032-59-9)	–	1.73
2-(1-Methylethoxy)phenyl methylcarbamate (propoxur, CAS no: 114-26-1)	–	0.14
2,2-Dimethyl-2,3-dihydro-7-benzofuranyl N-methylcarbamate (carbofuran, CAS no: 1563-66-2)	–	2.32
4-Methylthio-3,5-xylyl methylcarbamate (methiocarb, CAS no: 2032-65-7)	–	3.18

ologies [7,14–17]. Capillary or microLC using focalization or peak compression has been used for the determination of triazines in water using in-tube solid phase microextraction coupled to capillary liquid chromatography [13]. Chlorophenoxy acids have been determined in apple juice [14] and urine [16] using high injection volumes and focusing on the head of the capillary column. Trace amounts of heterocyclic amines have also been determined in cooked ham [15], smoked salmon and soft cheese [17] by cLC, again with high injection volumes and on-column focusing. Most of these separations have been carried out using C_8 or C_{18} packed columns.

Peak dispersion deteriorates selectivity and sensitivity of separation methods. Peak volumes are directly related to the square of the column diameter; therefore, the effect of band broadening sources becomes more evident with smaller columns. Extracolumn band broadening can compromise separations and it is one of the major challenges in miniaturized LC. The dispersion caused by pre-column components, such as sample injectors, column selection valves, column inlet filters and connecting tubing can produce a wide initial sample zone with a large dispersion that severely degrades resolution. Benefits of on-column concentration are available only if dispersion is minimized without peak height reduction.

The dispersion caused by injection of high volumes of sample can be expected to be dependent on many parameters, including injection volume, composition of injection mixture, pH and nature of the analytes injected. Multivariate statistical techniques have been employed frequently for the optimization of chromatographic systems [18]. All methods require the user to supply minimum and maximum values for each factor that defines the experimental domain to be investigated during the optimization procedure. Multivariate optimization of chromatographic systems can be carried out using the following procedure:

(i) Choose a statistical design to investigate the experimental region of interest.
(ii) Perform the experiments in random chronological order.
(iii) Perform analysis of variance (ANOVA) on the regression results so that the most appropriate model can be used to represent the data.

This paper presents an optimization study of focusing conditions for several compounds at trace levels, including easily ionisable compounds and acidic compounds with low log P (Table 1). Elution conditions studied are: isocratic and isothermal elution, gradient and isothermal elution, and isocratic and thermal gradient. The effect of an increase in sample volume on peak width (in terms of values of N) and on peak area has been evaluated. The band-spreading effects associated with large sample injection volumes are most pronounced for early-eluting peaks, since they have the smallest volume (narrowest peaks); for this reason, these peaks have been used for optimization of sensitivity (expressed as peak area) and performance (values of N). In this study, nine chlorophenoxy acids in their acid and ester forms, five carbamates and four heterocyclic aromatic amines were used due to their low log P and their ionisable character (Table 1).

2. Materials and methods

2.1. Chemicals and reagents

All reagents and solvents were of analytical grade and purified water from a Milli-Q system was used in all procedures (Millipore, Bedford, MA, USA). Methanol and acetonitrile of gradient HPLC quality were supplied by Scharlau (Barcelona, Spain). Chemicals including ammonium acetate, sodium hydroxide, phosphoric acid (85% pure), sulphuric acid (96% pure) and hydrochloric acid (35% pure) were purchased from Panreac (Barcelona, Spain). Acetic acid (99%) was obtained from Sigma (Steinhem, Germany).

Pesticides studied were: 2,4-D (99% pure), MCPA (95% pure) and 2,4,5-TP (97% pure) supplied by Aldrich; 2,4-D-1-methyl ester (97% pure), 2,4-DP (95% pure) and 2,4-DB (97% pure), from Sigma; MCPB (99% pure), 2,4-D-1-butyl ester (98.3% pure), MCPP (99% pure) and carbaryl (99.7% pure), from Riedel-de-Häen; aminocarb (98% pure), propoxur (99% pure), carbofuran (99% pure) and methiocarb (98.5% pure), from Chem Service (Table 1).

Heterocyclic amines studied were 1-methyl-9H-pyrido[3,4-b]indole (harman), purchased from Fluka (Buchs, Switzerland); 9H-pyrido[3,4-b]indole (norharman) from Sigma (Steinhem, Germany); 7-methoxy-1-methyl-9H-pyrido[3,4-b]indole (harmine) purchased from Sigma–Aldrich (Schnelldorf, Germany); and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), provided by Toronto Research Chemicals (Toronto, Canada) (Table 1). According to the manufacturers, the chemical purity of the synthetic reference compounds was higher than 98%.

2.2. Preparation of standard solutions

Stock solutions of chlorophenoxy acid herbicides were prepared by dissolving 20 mg of each one in 100 mL of methanol, while those of carbamates were prepared by dissolving 10 mg of each one in 10 mL of acetonitrile and then diluting to 50 mL with purified water.