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Superheated water as an eluent for reversed-phase high-performance liquid chromatography

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Abstract

Superheated/subcritical water can be used as an eluent in reversed-phase high-performance liquid chromatography as an alternative to methanol–water or acetonitrile–water mixtures. Mixtures of phenols, parabens, barbiturates and other analytes have been separated on polystyrene–divinylbenzene (PS–DVB) and ODS-bonded silica columns at temperatures up to 210°C and moderate pressures, with no degradation of the samples. The solvent presents virtually no disposal costs or purchase costs and is an environmentally friendly and cheap eluent. © 1997 Elsevier Science B.V.

Keywords: Mobile phase composition; Superheated water; Water, superheated; Phenols; Barbiturates; Parabens

1. Introduction

Supercritical fluid chromatography (SFC) using carbon dioxide based eluents has received considerable interest over the last 10 years [1] but apart from early studies, mainly using alkanes or nitrous oxide, there has been little interest in alternative eluents. One problem has been that many of the alternatives, such as sulphur hexafluoride, are chemically reactive or require excessive pressures or temperatures to become supercritical. Among the many attractions of carbon dioxide is that it is readily available, relatively cheap, non-toxic and causes no significant problems with disposal. These separations are effectively carried out in the normal-phase mode in which retention is determined by a combination of solvent polarity and volatility.

Recently, Hawthorne and co-workers [2,3] have demonstrated that supercritical water at 400°C and 350 bar and even subcritical water at 250°C and 50 bar can be used as an alternative solvent for the extraction of non-polar analytes, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), from soil and sediment samples. Under these conditions water has a low polarity and viscosity [4], and behaves as a typical low-polarity organic solvent similar to hexane. However, supercritical water is a reactive medium with the potential to promote oxidation or degradation of some solutes and has even been used to destroy toxic waste [5]. Potentially water has similar advantages as a clean solvent to carbon dioxide, with minimal purchase and disposal costs, but without the complications of being a compressed gas or for the need to condense it before pumping.

Typical reversed-phase liquid chromatography (RP-LC) systems make use of a hydrophobic stationary phase in combination with an aqueous mobile phase comprising a nominal fraction of a miscible organic solvent, such as methanol or acetonitrile. In RP-LC it is traditionally viewed that the eluent strength increases with decreasing mobile phase polarity. Water at ambient temperatures is thus deemed to be the weakest common chromatographic

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solvent because of its relatively high polarity (dielectric constant, ε=80 at 20°C). However, following the work of Hawthorne and coworkers [2,3], it was realised that at intermediate subcritical but elevated temperatures and pressures, pure water should behave as an eluent with a similar polarity to the mixtures of methanol and water that are typically employed as eluents in the RP-LC of moderately polar and non-polar analytes.

Although other studies have examined the effect of temperature on RP-LC [6–9], the composition of the aqueous–organic eluent has usually been held constant. Under these conditions the interest has been to observe the changes in the retention factors and separation efficiencies of the analytes rather than reducing the proportions of the organic modifier used. Some work has reported the use of a pure water mobile phase as an eluent in RP-LC [10,11] but these efforts have primarily focused on the quantitative determination of k_w retention factors as part of QSAR or prediction studies. Usually very short columns were used to compensate for the large retention factors. Recently Foster and Synovec [12] have developed a trifluoropropylsiloxane stationary phase based on a gas chromatographic material that could be used with a pure water mobile phase. They achieved reasonable retention factors for a range of aromatic analytes by substantially decreasing the phase ratio of the stationary phase relative to the mobile phase volume.

The present work examines the application of superheated water, in the absence of an organic modifier, as a medium polarity eluent for reversed-phase chromatography as an alternative to aqueous–organic modifier eluents. The effect of different operating temperatures and columns have been examined. The eluent has no absorbance down to 190 nm and it should be possible to detect even weak chromophores. A preliminary description of this work has been reported [13,14], which demonstrated the application of superheated water as an eluent for a range of analytes, including pharmaceuticals and phenols, on a polystyrene–divinylbenzene (PS–DVB) column at temperatures up to 210°C. These presentations also proposed that it should be possible to use common GC detectors, such as the flame ionisation detector with the aqueous eluent and that temperature programming of the column could be used to generate a gradient elution as the polarity of the eluent would systematically decrease. Since the present paper was submitted for publication, Miller and Hawthorne [15] have reported the use of flame ionisation detection and temperature programming for a range of analytes, including alcohols, phenols and amino acids on a narrow bore PS–DVB column.

2. Experimental

2.1. Reagents

Water was triply deionised and treated through a Elga Maxima HPLC purification unit (Elga, Wycombe, UK) at an output of 18.2 MΩ. Acetonitrile and methanol were of HPLC-grade from Fisons (Loughborough, UK). Test compounds were of laboratory-grade from a range of suppliers.

2.2. Chromatographic conditions

The high temperature chromatograph consisted of a Jasco PU 980 HPLC pump (Hachioji, Tokyo, Japan), a Rheodyne Model 7125 injector (Cotati, CA, USA) fitted with a 20 μl loop, a Pye Unicam series 104 GC oven controlled isothermally with a Pye Unicam oven programmer, a Jasco 875 UV–Vis detector at 254 nm and a Jasco 880/81 back pressure regulator maintained at 20 bar. A 1 m×0.01 in. (1 in. 2.54 cm) I.D. length of stainless-steel tubing was placed in the oven between the injection valve and the column as a pre-heating coil. The mobile phase was purged with nitrogen to deoxygenate the mobile phase, thereby helping to prevent any solute oxidation or corrosion to the chromatographic system.

Liquid chromatography was carried out using a Pye Unicam 4015 pump, a Pye Unicam 4025 variable UV–Vis detector at 254 nm and a Jones 7960 block heater at 30°C with a Rheodyne 7125 manual injector and a 20 μl loop.

The columns used in these studies was packed with PLRP-S (5 μm PS–DVB) (150 mm×4.6 mm I.D., Polymer Labs., Shropshire, UK) or with Spherisorb ODS2, 5 μm, (150 mm×4.6 mm I.D., Phase Separations, Clwyd, UK).

3. Results and discussion

3.1. Separations on a PS–DVB column

As octadecylsilyl (ODS)-bonded silica based columns have been reported to be unstable when used at high temperatures [16], a PS–DVB column was selected for the initial studies. This column has been reported to operate satisfactorily at up to 80°C [7] and similar materials are frequently used in size-exclusion polymer analysis at temperatures up to 160°C. Previous studies in this and other laboratories have found that although this column shows a strong retention for non-polar analytes, polar analytes are rapidly eluted [17].

The column was examined initially at a temperature of 40°C with an acetonitrile–water (5:95) eluent. Under these conditions benzamide was eluted with a short retention factor ($k=1.43$) and a good peak shape. On reducing the acetonitrile content to 1% and ultimately to pure water (0% acetonitrile), the retention factor of benzamide increased progressively to $k=9.66$. If the temperature was then increased to 60°C, with pure water as the mobile phase, the retention was reduced to $k=6.58$. Further increases in temperature to 150°C decreased the retention factor for benzamide still further to $k=1.56$, close to the retention in the acetonitrile–water (5:95) mixture. For temperatures above the boiling point of water, a small back pressure (20 bar) was applied to the column using an electronically controlled back-pressure regulator designed for SFC.

A wider range of analytes was then examined, ranging from relatively polar amides (Fig. 1) and phenols to less polar ketones and aldehydes (Fig. 2), and the effect of increasing the temperature above 100°C was studied with water alone as the eluent. In each case the analytes were eluted with good peak shapes. For each compound studied, there was a systematic decrease in retention factor with increasing temperature from 150°C to 180°C (Table 1). However, unlike previous studies of the effect of temperature changes in conventional reversed-phase HPLC [8], plots of $1/T$ against retention factor for the phenolic compounds were not linear (Fig. 3). Fitting the data points to a quadratic function displayed an excellent fit with correlation coefficients of 0.9979, 0.9991 and 0.9998 for phenol, *m*-methoxyphenol and *m*-cresol, respectively. This suggested that additional factors, such as the change in dielectric constant of the eluent, were having a significant effect in addition to the conventional ΔH effects.

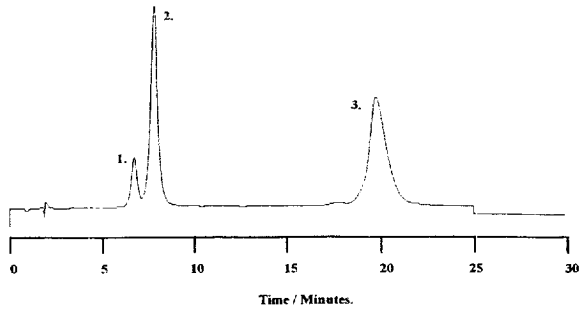

Fig. 1. Chromatogram of arylamides on a PS–DVB column. Conditions: column, PLRP-S, mobile phase, 100% water at 1 ml/min, detection, 254 nm, temperature 140°C. Peaks: (1) benzenesulphonamide, (2) benzamide, (3) *m*-toluamide.

The high temperature separation of the phenols showed decreasing retention with increasing temperature, with baseline resolution achievable at all the temperatures studied. These results were compared with the separation of the same analytes under conventional reversed-phase conditions using a acetonitrile–water (20:80, v/v) mobile phase at room temperature [14]. These results confirmed that high-temperature water could behave as a eluent comparable to conventional acetonitrile–water mixtures.

There have been a number of conflicting reports on whether high temperature operation in RP-LC has a beneficial or negative effect on the efficiency of the chromatographic separation [18]. It is generally

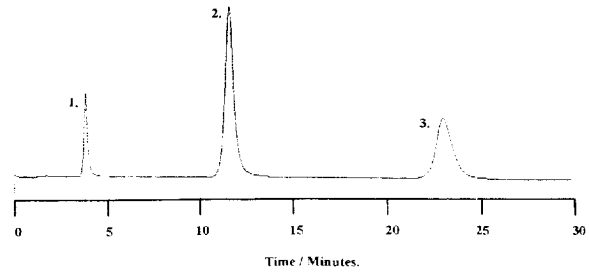

Fig. 2. Chromatogram of alkyl aryl ketones on a PS–DVB column. Conditions: column, PLRP-S; mobile phase, 100% water at 1 ml/min, detection, 254 nm, temperature 200°C. Peaks: (1) *m*-hydroxyacetophenone, (2) acetophenone, (3) *m*-methylacetophenone.

Table 1
Retention factors of a range of analytes on a PS–DVB column on elution with superheated water at increasing temperatures

Compound	Retention factors (k) Temperature			
	150°C	160°C	170°C	180°C
Phenol	3.20	2.59	2.01	1.68
m-Methoxyphenol	5.15	3.98	2.98	2.39
m-Cresol	9.32	7.09	5.33	4.21
Benzenesulphonamide	1.24	0.96	0.74	
Benzamide	1.56	1.22	0.96	0.27
m-Toluamide	4.76	3.55	2.73	2.16
m-Hydroxybenzaldehyde	2.82	2.07	1.44	1.17
m-Tolualdehyde	(k>30)	(k>30)	25.57	18.24
m-Hydroxyacetophenone	3.56	2.93	2.31	1.83
Acetophenone	28.78	20.72	15.26	11.2

Separation conditions: column, PLRP-S; eluent, water; flow-rate, 1 ml/min; column temperatures as Table.

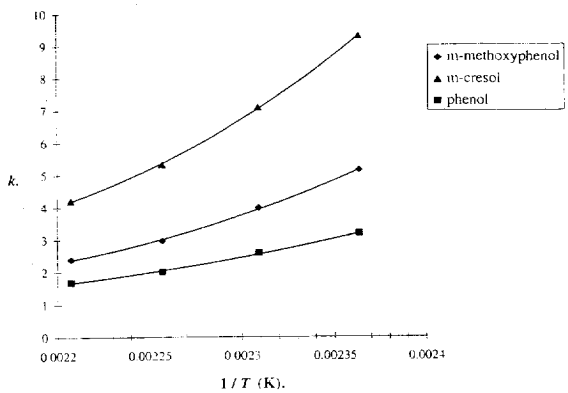

Fig. 3. Relationship between retention factors and temperature for the separation of a series of phenols on a PS–DVB column. Conditions: column, PLRP-S; mobile phase, 100% water at 1 ml/min.

predicted that an increase in column temperature should improve column efficiency, due to a reduction in eluent viscosity and an increase in solute diffusivity [9,19]. The reported dynamic viscosity of water decreases markedly over the temperature range studied, from $0.54 \cdot 10^{-3}$ kg/ms at 50°C to $0.15 \cdot 10^{-3}$ kg/ms at 180°C [20]. However, it appeared that there were no significant changes in the efficiency for two typical phenols over this temperature range (Table 2). However, there was a noticeable improvement in the asymmetry factor for *m*-cresol, whereas for phenol the asymmetry factor deteriorated. It seemed that as the temperature increased, the increase in diffusion rate reduced the influence of the C terms in the van Deemter equation but increased the influence of the B term. Further studies will determine the effect of temperature on the relationship between the

Table 2
Efficiency (N) and asymmetry factors ($As_{0.1}$) for phenols on a PS–DVB column on elution with superheated water at increasing temperatures

Compound	Column temperature							
	150°C		160°C		170°C		180°C	
	N	$As_{0.1}$	N	$As_{0.1}$	N	$As_{0.1}$	N	$As_{0.1}$
Phenol	1799	2.17	1927	2.30	1913	2.34	1685	2.57
m-Cresol	2407	1.72	2613	2.18	2484	1.89	2292	1.17

Separation conditions: column, PLRP-S; eluent, water; flow-rate, 1 ml/min; column temperature as Table. Asymmetry measured at 10% of peak height.

height equivalent to a theoretical plate (H) and the linear flow velocity (u).

Over the temperature range studied, the density of water shows only a small change from 0.96 g/cm^3 at 100°C to 0.86 g/cm^3 at 200°C [21]. Thus unlike the significant influence of pressure on carbon dioxide in SFC, the elution properties of superheated water are expected to be virtually unaffected by pressure. The applied back-pressure on the column will only need to be sufficient to prevent boiling of the eluent. The vapour pressure of water only rises to about 15 bar at 200°C [21] and thus much lower pressures are needed compared to conventional SFC using CO_2; consequently making the overall system more robust. In addition as the water eluent is pumped at room temperature there are no requirements, as with carbon dioxide, to cool the pump head.

A series of paraben (alkyl *p*-hydroxybenzoate) homologues were separated at 210°C in the order of increasing chain length methyl (k=1.28), ethyl (k=2.72), propyl (k=5.79) and butyl (k=11.95) and demonstrated that superheated water could resolve analytes on the basis of their hydrophobicities. This confirmed that the retention in this system was governed by a reversed-phase mode of separation. No degradation or hydrolysis of the ester groups or column material was observed [14]. The reduced reactivity of the water under these conditions compared to lower temperatures is presumably a function of its reduced polarity.

The applicability of this approach to pharmaceuticals has also been demonstrated by the separation of a mixture of five barbiturates at 200°C [13,14]. However, the elution order differed from that observed with a conventional reversed-phase HPLC performed on an ODS-bonded silica column [22] or in SFC studies using a PS–DVB column with carbon dioxide as the mobile phase [23].

3.2. Separations on an ODS-bonded silica column

A series of studies was also carried out using an ODS-bonded phase silica column. These columns were expected to be less stable [16] but good results were obtained. Again there was a ready elution of relatively non-polar analytes from the column and the retentions decreased with increasing temperature (Table 3). Noticeably, the analytes could be eluted from the ODS silica column at considerably lower temperatures (≈<40–50°C) than from the PS–DVB column. In a typical separation (Fig. 4) three phenols were separated within 10 min at 120°C. Again the homologous series of parabens were eluted in the order of increasing chain length (Fig. 5) but required only 140°C compared to 210°C on the PS–DVB column for similar retention times. The weaker retentions on the ODS-column agree with the conventional reversed-phase separation at room temperature when lower proportions of organic modifier were usually needed for the equivalent retentions for an PS–DVB column [17].

However, recent work suggested that prolonged

Table 3
Retention factors of a range of aromatic analytes on an ODS-bonded silica column on elution with superheated water at increasing temperatures

Compound	Retention factors (k) Temperature			
	100°C	110°C	120°C	130°C
Phenol	2.63	2.17	1.61	1.22
m-Methoxyphenol	4.51	3.54	2.61	1.87
m-Cresol	7.64	6.08	4.55	3.31
Benzenesulphonamide	1.41	1.09	0.87	0.63
Benzamide	2.28	1.78	1.42	1.05
m-Toluamide	7.59	5.70	4.36	3.09
m-Hydroxybenzaldehyde	2.63	1.98	1.52	1.04
m-Tolualdehyde	>20	>20	17.29	12.07
m-Hydroxyacetophenone	2.70	2.04	2.75	1.94
Acetophenone	>20	15.87	10.37	7.59

Separation conditions: column, Spherisorb ODS2; eluent, water; flow-rate, 1 ml/min; column temperature as Table.

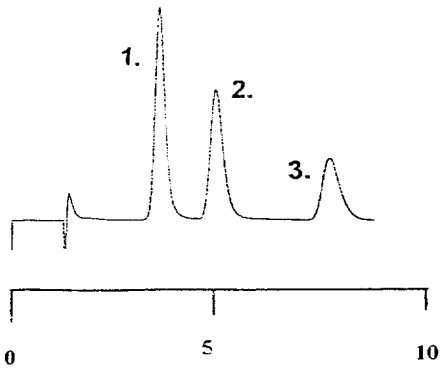

Fig. 4. Chromatogram of phenolic compounds on ODS-bonded silica column. Conditions: column, Spherisorb ODS2; mobile phase, 100% water at 1 ml/min, detection, 254 nm, temperature 120°C. Peaks: (1) phenol, (2) *m*-methoxyphenol, (3) *m*-cresol.

use of the Spherisorb ODS2 columns may result in some loss of retention and suggests that the stability of these silica based bonded phases may be limited. Further work is in progress to determine the long term stability and efficiency of different brands of reversed-phase material under these conditions and to examine the effects of different flow-rates and the presence of additives such as buffers to the eluent. Additional studies will examine the proposition that as temperature programming will result in a decrease in polarity of the eluent, the effect should be similar to a gradient elution.

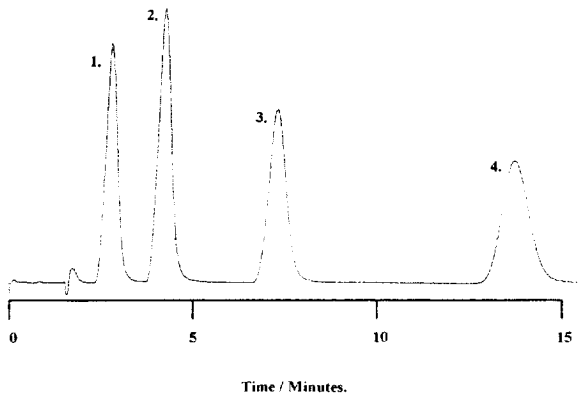

Fig. 5. Chromatogram of a mixture of paraben homologues on ODS-bonded silica column. Conditions: column, Spherisorb ODS2; mobile phase, 100% water at 1 ml/min; oven temperature, 170°C. Peaks: (1) methyl paraben, (2) ethyl paraben, (3) propyl paraben, (4) butyl paraben.

4. Conclusions

The use of superheated water as an organic free eluent in RP-LC using PS–DVB and ODS-bonded phase columns has been demonstrated. The elution order of analytes corresponded to a reversed-phase separation and in some cases gave enhanced separations and shorter analysis times compared with conventional reversed-phase HPLC using water–organic solvent mobile phases. Work is currently under way to determine the retention mechanism and selectivity of this superheated water system, and to investigate the stability of these and other packing materials that may be amenable to this approach.

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