An Investigation into the Effect of Chemical and Physical Polymer Structure on Reverse Phase Extraction of Small Molecules such as Paracetamol and Haloacetic Acids

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A series of clean-up columns for reverse phase solid-phase extraction (SPE) were packed with various polymeric resins with different chemical structure, surface properties, hydrophobicity and porosity. These SPE columns were used for the isolation and concentration of paracetamol and haloacetic acids by reverse phase chromatography. Results have demonstrated a strong effect of pore size on the recovery, showing that low pore size is beneficial. The newly-developed Purolite PuroPhase™ Chromalite® resins show good levels of recovery compared to similar commercially available resins and confirm the effect of pore size on recovery of small organic molecules.

Introduction

An analytical method for forensic casework requires an extremely high degree of specificity in addition to the more universal requirements of sensitivity, precision, and linearity. The results shown in this article demonstrate that the selection of resins used for Solid Phase Extraction, particularly in relation to their chemical and physical make-up, has a significant effect on the final results [1, 2].

Solid phase extraction has developed into one of the more powerful tools for clean-up and concentration of very low levels of analytes from complex matrices, especially when paired with sensitive detection methods such as HPLC and MS technologies [3, 4, 5]. In recent years automation has significantly increased the efficiency of this technique, allowing a high-throughput approach to sample testing. By careful selection of solid phase extraction sorbents, paying attention to the polymer structure, the efficiency of clean-up and concentration activities can be improved well beyond the original capabilities of early methods [6, 7, 8].

The levels of pharmaceutical compounds leaching into drinking water supplies have become a concern and regular testing to confirm the safety and levels of contamination is now widespread [9, 10]. Paracetamol is one of the most commonly used and readily available analgesic drugs and therefore one of those most suspected to be present as an environmental contaminant. Although paracetamol is very safe at recommended dosage rates, there are still a significant number of cases of intoxication and fatalities due to paracetamol overdose, an estimated 32,000 overdoses annually in England and Wales alone, with a fatality rate of 0.4% [11] and the long-term effects of low-level exposure are not thoroughly understood. For this reason, good detection of paracetamol is essential, hence the selection of this compound for study.

The wide range of resins for solid phase extraction was also tested in the clean-up and extraction of haloacetic acid. Haloacetic acids (HAAs) are widespread environmental pollutants and, due to their environmental impact, there is a growing interest in their determination in aqueous solutions. HAAs are chemical byproducts of water chlorination and chloramination, to control infectious microbial contaminants [12], however the possibility exists for the chemicals used to subsequently degrade into HAAs. HAAs are highly water-soluble and are toxic to humans, plants and algae [13, 14], hence their accurate detection is again important to use in monitoring drinking water supplies. Due to the interest in improving the efficiency of detection of both of these classes of compounds, examples were selected from both compound classes.

Keywords

Solid Phase Extraction; Sample Preparation; Chromalite; Sorbents; Polymer-Based Sorbents; Water Analysis; reverse phase

Experimental

SPE Separation - Paracetamol

Pre-packed cartridges for each sorbent were obtained for testing. 500 mg bed mass, 6 mL cartridges were obtained for Waters Oasis HLB (due to no availability of 200 mg, 3 mL cartridge), all others including Purolite PuroPhase were 200 mg bed mass, 3 mL. The cartridges were attached to an Interchim 6.25ws SPE machine and the extraction was performed automatically. First, the sorbent was conditioned using 7 mL methanol followed by 7 mL water, both applied at a flow rate of 15 mL / min. The sorbent was not allowed to dry prior to the application of 1 mL of a 0.1 mg / mL solution of paracetamol in water at a flow rate of 2 mL / min. After a 10 second pause, the sorbent was then dried by nitrogen flow for 30 seconds prior to elution with 3 applications of 2 mL methanol at a flow rate of 2 mL / min.

The eluted fraction was then evaporated and the residue dissolved in 5 mL water prior to HPLC analysis.

Table 1: Physical characteristics of the resins.

Resin	Polymer description	Particle Size (µm)	Surface Area (m^2/g)	Avg. Pore Diameter (Å)
Chromalite PCG600M	Macroporous poly-DVB	70-80	>700	140
Chromalite 70MN	Hyper-crosslinked microporous polystyrene	60-80	>1500	30
Chromalite PCG900M	Macroporous poly-DVB	70-80	>600	250
Chromalite PCG1200M-HEMA	Macroporous hydroxyethyl meth-acrylate – DVB copolymer	50-100	>500	300
Chromalite PCG1200CPlus	Macroporous poly-DVB	100-110	>800	320
Chromalite PCG1200M	Macroporous poly-DVB	70-80	>600	400
Macherey-Nagel HR-P	Styrene/DVB	50-100	1200	60
Phenomenex Strata SDB-L	Styrene/DVB	100	500	260
Mitsubishi Diaion HP20SS	Styrene/DVB	63-150	560	580
Merck Lichrolut EN	Ethylstyrene/DVB	40-120	1200	60
Waters Oasis HLB	DVB/N-vinyl-pyrrolidone	60[1]	800	80
Agilent Bond-Elut ENV	Styrene/DVB	125	700	450

[1] *The 6 mL syringe used was packed with 500 mg sorbent of a 60 µm particle size bead. A comparable 3 mL syringe would be packed with 100 mg* sorbent with 30 um particle size bead [23].

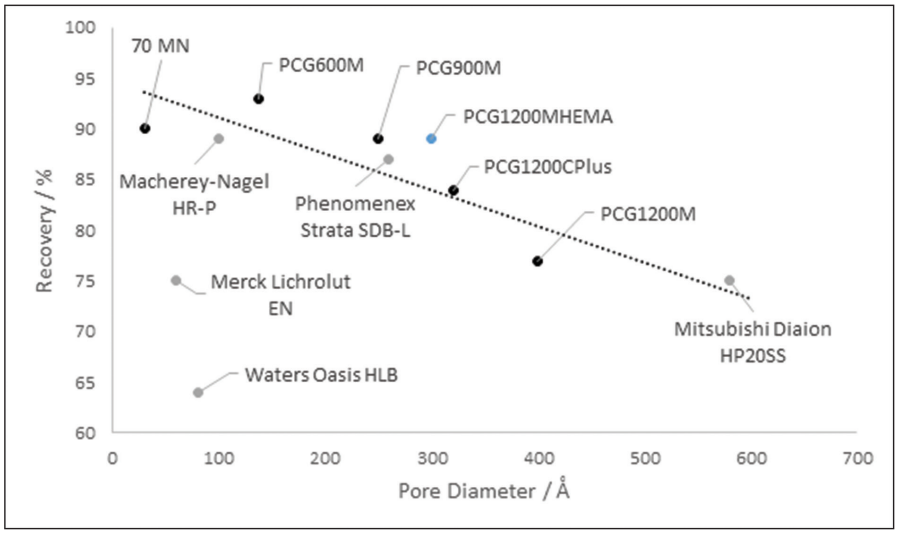

Figure 1: The relationship between paracetamol recovery and pore diameter for the tested resins. Agilent Bond-Elut ENV result not shown for clarity; trendline based on Purolite Chromalite sorbents (excluding PCG1200MHEMA) only.

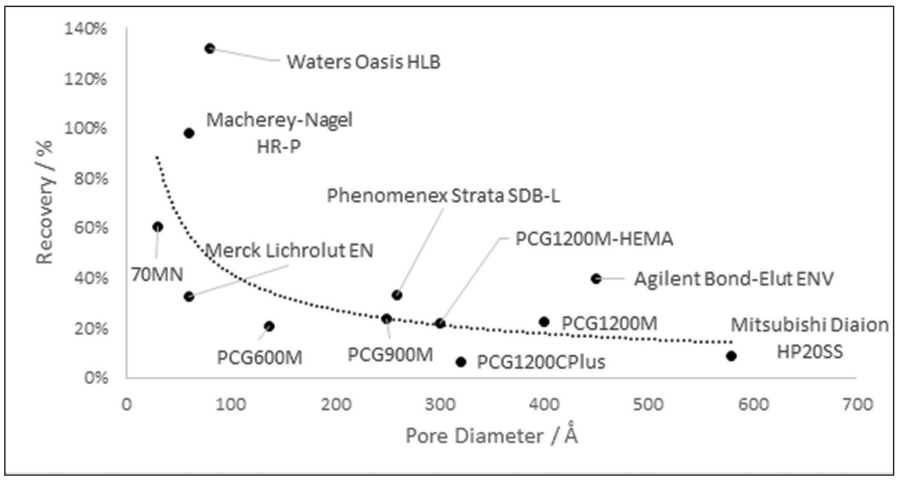

Figure 2: The relationship between recovery of bromochloroacetic acid (BCAA) and pore diameter. Trendline drawn including all tested resins, coefficient of determination 59%.

HPLC Quantification - Paracetamol

HPLC was performed using a Perkin-Elmer Flexar HPLC system, with detection by UV absorption at 220 nm. The mobile phase used was a 70:20:10 mix of water:acetonitrile:20 mM ammonium acetate in water, with a final pH of mobile phase of 7.0. The mobile phase was run at 1 mL / min for 6 minutes isocratically at 25°C. A calibration curve was generated from an external standard (Sigma-Aldrich) for paracetamol to allow the quantification of the recovery levels of paracetamol.

SPE Separation – Haloacetic Acids

Pre-packed cartridges for each sorbent were obtained for testing. 500 mg bed mass, 6 mL cartridges were obtained for Waters Oasis HLB (due to no availability of 200 mg, 3 mL cartridge), all others including Purolite PuroPhase were 200 mg bed mass, 3 mL. The cartridges were attached to an Interchim 6.25ws SPE machine and the extraction was performed automatically. The sorbents were first conditioned using 5 mL methanol followed by 3 mL water (acidified to pH 2.5 with sulphuric acid), both applied at a flow rate of 1 mL / min. The sorbent was not allowed to dry prior to the application of 5 mL of a haloacetic acid mix containing 4 µg / mL in water of six haloacetic acids (mono-, di- and tri- chloroacetic acids, mono- and di-bromoacetic acids and bromochloroacetic acid) at a flow rate of 5 mL / min. The sorbent was then washed with 1 mL water (pH 2.5) at a flow rate of 1 mL/min prior to elution with 2 applications of 2 mL of a mixture of 1:3.5:3.5 water:methanol:acetone at a flow rate of 1 mL / min.

Table 2: Paracetamol and haloacetic acid recoveries

Resin	Recovery of Paracetamol	Recovery of Haloacetic Acid[1]				
		MCAA	MBAA	BCAA	DBAA	TCAA
Chromalite PCG600M	93 % ± 5 %	40 % ± 6 %	36 % ± 15 %	21 % ± 5 %	36 % ± 8 %	49 % ± 3 %
Chromalite 70MN	90 % ± 4 %	70 % ± 19 %	53 % ± 7 %	61 % ± 7 %	75 % ± 10 %	82 % ± 7 %
Chromalite PCG900M	89 % ± 2 %	52 % ± 27 %	37 % ± 13 %	23 % ± 3 %	43 % ± 4 %	56 % ± 3 %
Chromalite PCG1200M-HEMA	89 % ± 2 %	55 % ± 9 %	45 % ± 7 %	22 % ± 4 %	39 % ± 7 %	34 % ± 10 %
Chromalite PCG1200CPlus	84 % ± 2 %	95 % ± 6 %	60 % ± 4 %	6 % ± 3 %	9 % ± 2 %	6 % ± 5 %
Chromalite PCG1200M	77 % ± 7 %	43 % ± 10 %	35 % ± 6 %	22 % ± 1 %	37 % ± 11 %	42 % ± 5 %
Macherey-Nagel HR-P	89 % ± 1 %	41 % ± 4 %	30 % ± 4 %	98 % ± 46 %	103 % ± 43 %	107 % ± 42 %
Phenomenex Strata SDB-L	87 % ± 1 %	26 % ± 14 %	22 % ± 16 %	33 % ± 7 %	62 % ± 10 %	42 % ± 10 %
Mitsubishi Diaion HP20SS	75 % ± 4 %	87 % ± 8 %	62 % ± 4 %	9 % ± 1 %	12 % ± 4 %	16 % ± 2 %
Merck Lichrolut EN	75 % ± 3 %	44 % ± 5 %	27 % ± 2 %	32 % ± 2 %	46 % ± 2 %	40 % ± 4 %
Waters Oasis HLB[2]	64 % ± 3 %	16 % ± 5 %	11 % ± 3 %	132 % ± 4 %	92 % ± 2 %	54 % ± 2 %
Agilent Bond-Elut ENV	29 % ± 8 %	23 % ± 3 %	16 % ± 2 %	40 % ± 1 %	82 % ± 8 %	40 % ± 2 %

MCAA = monochloroacetic acid, MBAA = monobromoacetic acid, BCAA = bromochloroacetic acid, DBAA = dibromoacetic acid, TCAA = trichloroacetic acid.
[2] 500 mg, 6 mL cartridge used for Waters Oasis HLB. All other cartridges were 200 mg, 3 mL.

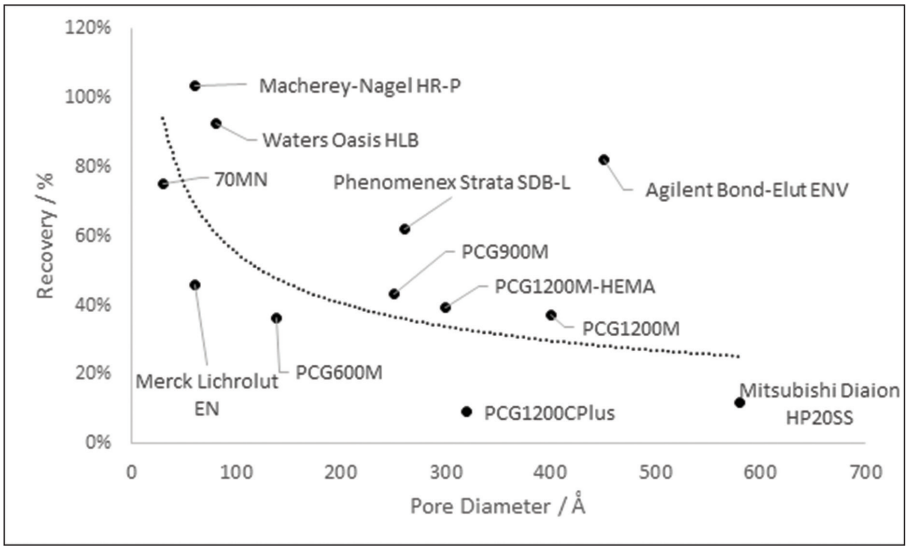

Figure 3: The relationship between recovery of dibromoacetic acid (DBAA) and pore diameter.

Trendline drawn including all tested resins, coefficient of determination 46%.

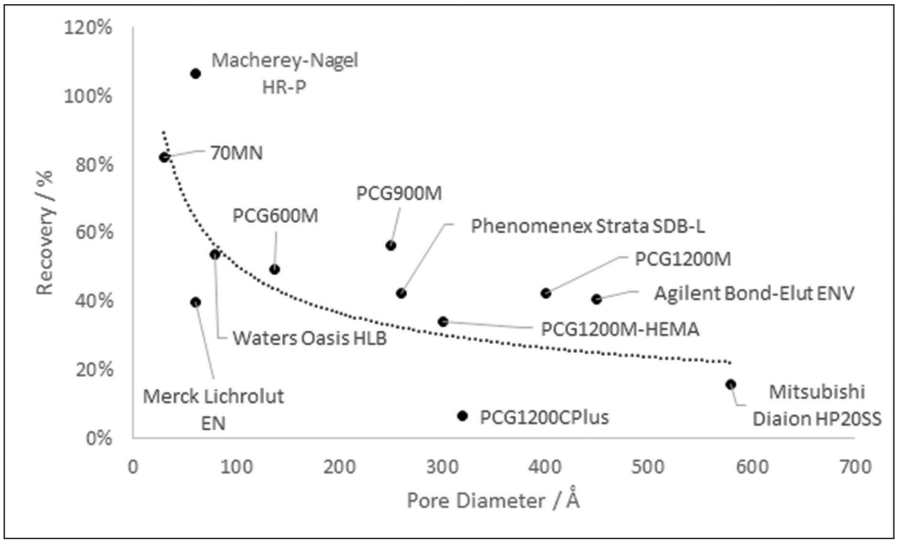

Figure 4: The relationship between recovery of trichloroacetic acid (TCAA) and pore diameter.
Trendline drawn including all tested resins, coefficient of determination 40%.

HPLC Quantification – Haloacetic Acids

HPLC was performed with a modified literature method [16] using a Perkin-Elmer Flexar HPLC detection system, with detection by SofTA 300S ELSD. The mobile phases used were a) water containing 5 mM di-N-butylamine, pH 5.5 with acetic acid and b) acetonitrile containing 5 mM di-N-butylamine. The mobile phase was run at 1 mL / min at a gradient from 95% A to 75% A over 20 minutes. A calibration curve was generated from an external standard (Thames Restek) to allow the quantification of the recovery levels except for dichloroacetic acid, which is obscured by a sulphuric acid peak. ELSD spray chamber was at 25°C and drift tube at 55°C.

Results and Discussions

A study on Solid Phase Extraction of paracetamol and haloacetic acids was performed on a series of different adsorbents with different chemical and physical characteristics as reported in Table 1.

All polymers reported in Table 1 are hydrophobic due to the presence of divinylbenzene (DVB) in their structure. Purolite Chromalite® PCG polymers are characterised by different pore sizes ranging from about 140 to 400 Å. Chromalite 70MN, Macherey-Nagel HR-P [17, 18], Merck Lichrolut EN [19, 20] are characterised by very high surface area (> 1000 m2/g) and low pore sizes (< 100Å).

Chromalite PCG1200MHEMA and Waters Oasis HLB [21, 22] are more hydrophilic than the other resins listed in Table 1 due to the presence in the polymer structure of hydroxyethyl-methacrylate and N-vinyl-pyrrolidone respectively.

A systematic study to understand the effect of physical and chemical characteristics of the polymer backbone on the interaction with different molecules was performed using paracetamol and haloacetic compounds.

In both the paracetamol and haloacetic acid experiments, the recovery was attempted using relatively hydrophobic SPE resins, hence improved by maintaining a low level of dissociation of the analytes.

For paracetamol, this low level of dissociation requires maintaining a pH below the pKa of 9.5, which is achieved in the SPE by using water as the solute and maintained more closely in the HPLC analysis by the use of a buffered mobile phase to ensure that the pH is kept at 7.0.

The haloacetic acids required a more nuanced approach, as the pKa values vary from 0.7 to 2.9. For the SPE of the haloacetic acids, water acidified to pH 2.5 was used to condition the sorbent as this was the same as the pH of the sample to be applied. For the HPLC analysis, the use of an ion pair agent, di-N-butylamine, necessitated full dissociation of the HAAs, hence the mobile phase was set to pH 5.5, well above the pKa values to ensure good detection.

Recovery data for each resin (%) shown in Table 2 were calculated by comparing the UV signals from the elute fractions to those of the load solutions.

A relationship can be drawn between the porosity of all the adsorbents and the recoveries observed, which is shown in Figure 1 for paracetamol and in Figures 2, 3 and 4 for the different haloacetic acid compounds. As a general trend, the recovery of paracetamol is related to size of pores of polymers: lower pore size adsorbents give better recovery. When considering as an example Macherey-Nagel HR-P, Phenomenex Strata SDB-L [24], Mitsubishi Diaion HP20SS [25], Agilent Bond-Elut ENV [26], Chromalite PCG1200M, Chromalite PCG1200CPlus, PCG900M, PCG600M which have the same polymer backbone based on DVB, results show that when pore size decreases from 580 Å (Agilent Bond-Elut ENV) to 140 Å (PCG600M) the recovery yield increases from 29% to 93% respectively. The trend is almost linear as shown by dotted line in Figure 1.

[1]The recovery yield obtained with Waters Oasis HLB was only 64% despite a small pore size of 80 Å and an increased quantity of sorbent compared to the other tested resins, and this can be due to the strong interaction of vinyl-pyrrolidone with the paracetamol. This effect was not observed when using PCG1200MHEMA which contains hydromethyl-methacrylate in the polymer backbone. The higher recovery observed with PCG1200MHEMA (89%) may be due to the affinity of the resin for the amide or hydroxyl groups on the paracetamol, thus resulting in a higher level of recovery than would be predicted by the pore diameter.

Both Macherey-Nagel HR-P and Chromalite 70MN have a polymer structure characterised by a low porosity (60 and 30 Å respectively) but very high surface area (1200 and 1500 m2/g respectively) and these features seem to have a positive effect on recovery of paracetamol, with values of 89-90%.

The recovery analysis of haloacetic acids was performed similarly to paracetamol with the only difference that the quantification was done using ELSD signals from the five studied haloacetic acids in the elute fractions and comparing them with the load solution. Results are shown in Table 2.

Haloacetic acids are very small organic molecules and the porosity of polymers affects the recoveries. Large porosities (> 200 Å) do not provide in general good recoveries and this can be ascribed to the high diffusion and permeation of the molecules inside the pores and consequent more difficult elution.

For the mono-substituted haloacetic acids (MCAA and MBAA) there is no obvious trend related to porosity. PCG1200CPlus and Mitsubishi HP20SS resulted both in quite high recoveries for the MCAA and MBAA with recoveries higher than 60%.

For the bulkier di-substituted haloacetic acids, however, there is a correlation between recovery and pore diameter, this time non-linear, as shown in Figures 2 and 3.

With the exception of the Waters Oasis HLB and the Agilent Bond-Elut ENV, which show results above expected recovery based on porosity, the tested resins fit well to a power-based trend. This trend is also duplicated, although to a lesser extent, in the recovery of trichloroacetic acid as shown in Figure 4.

In this case, the Oasis HLB and Bond-Elut ENV are much closer to the general trend, suggesting that dibromoacetic acid is particularly well-recovered by both of these resins. The recovery levels in the recovery of the haloacetic acids indicates that differences in pore diameter for low pore diameters (below 100 Å) is even more crucial than for paracetamol, with an increase from 50 to 150 Å appearing to correspond to approximately 30% recovery. Beyond this initial section, however, the decrease in recovery is much less rapid, indicating that the limiting factor no longer applies.

Conclusions

A study of recovery of paracetamol and haloacetic acids has been conducted using different available commercial resins and comparing with the new Purophase products. All resins are used for solid phase extraction based on reverse phase extraction mode. Results obtained were analysed in relation to polymer structure and porosity. Paracetamol is a small molecule comprising a highly hydrophobic region due to the presence of aromatic ring and a hydrophilic region formed of amide and hydroxyl groups. The highest yields in recovery are obtained with polymers with highly hydrophobic structures (polystyrene or divinylbenzene) and small porosity.

Haloacetic acid mixtures are composed of very small molecules and in this case the polymer structures again play a key role in the recoveries showing that pore size < 100 Å is beneficial to achieve higher recovery yields. A strong effect of surface area is also highlighted, showing that when polymers have very large surface area (>1000 m^2/g) the recovery is higher.

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