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Simultaneous Extraction and Methylation of Acidic Analytes Adsorbed onto Ion Exchange Resins Using Supercritical Carbon Dioxide Containing Methyl Iodide

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Methylation of a wide range of organic acids with methyl iodide was simply and efficiently performed on anion exchange resins with either supercritical carbon dioxide or acetonitrile as solvents. Analytes including chlorophenoxyacetic acids, pentachlorophenol, and quinoxaline-2-carboxylic acid were displaced from the resin in a single step as their methyl esters or ethers in high yield using the supercritical fluid extraction (SFE) system. The conversion of 2,4-D and 2,4,5-T (solutions of 100 and 35 ppb, respectively) to their methyl esters was complete in 30 min and gave yields of 92% and 99% with coefficients of variation of 10%. Analytes in up to 200 mL of aqueous solution could be trapped on 0.1 g of AG MP-1 anion exchange resin and derivatized and eluted using methyl iodide in supercritical carbon dioxide at 200 bar and 80 °C. Less acidic compounds including albendazole, fenbendazole, triclabendazole, and sulfadimidine could also be derivatized on the resin under SFE conditions or in a quick and inexpensive procedure using acetonitrile; however, these compounds gave lower yields and multiple methylated products.

There has been increasing recent interest in the simultaneous derivatization and extraction of analytes by supercritical fluid extraction (SFE).[1–6] The preparation of less polar derivatives during the course of their extraction not only allows the extension of SFE to a wider range of compounds but at the same time facilitates the application of GC techniques to their detection and quantitation. In this paper a technique is described which allows methylation and SFE of highly polar organic acids trapped and concentrated from aqueous solutions onto an anion exchange resin.

We reported in a previous paper[7] the use of supercritical carbon dioxide as the nonaqueous solvent in the phase transfer methylation of 2,4-dichlorophenoxyacetic acid (2,4-D, **1**) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, **2**) with tetrahexylammonium hydrogen sulfate as the phase transfer catalyst. The high-yield direct derivatization of these analytes from aqueous solutions was achieved using simple equipment at moderate pressures. The mechanism of the reaction process was elucidated, and the addition of methanol as an SFE modifier was shown to be deleterious to the reaction. Two significant drawbacks of the phase transfer method were a 3-mL limitation on the volume of aqueous solution which could be used for simultaneous derivatization–SFE with the available equipment and the requirement to remove coextracted phase transfer reagent with SM-7 resin prior to GC determination.

The mechanism of methylation was shown to involve reaction of methyl iodide with the anionic form of the acids present in the supercritical carbon dioxide phase as ion pairs with the quaternary ammonium phase transfer catalyst. We therefore envisaged that a similar methylation process would be possible for the anion of an acidic analyte attached to the surface of a quaternary ammonium ion exchange resin, the only effective difference being that the resin procedure would involve a heterogeneous rather than a homogeneous reaction.

The use of ion exchange resins as heterogeneous esterification mediators in synthetic organic chemistry has been described,[8] but similar analytical applications have not been pursued. In these reactions, the acid is absorbed onto the resin as its anion and displaced as the ester by treatment with an organic halide. Yields are excellent with primary halides. We have previously used this principle in the development of an analytical method for determination of a family of thyrostatically active compounds, the thiouracils. These were absorbed onto a basic resin and converted into dimethylated derivatives suitable for GC determination by treatment with methyl iodide in acetonitrile.[9] Dimethylated thiouracils could be slowly eluted from the column of resin as they were formed, but as the rate of the methylation reaction was not rapid, a stop-flow technique was used to ensure an adequate contact time between the methyl iodide solution and the adsorbed thiouracils. With these precautions, final recoveries were satisfactory, and the method was considerably easier than alternative methylation procedures. The advantage of the process was the ability to significantly preconcentrate ionic analytes from aqueous solution on a small quantity of ion exchange resin and to eliminate nonionic impurities during the operation. The successful use of

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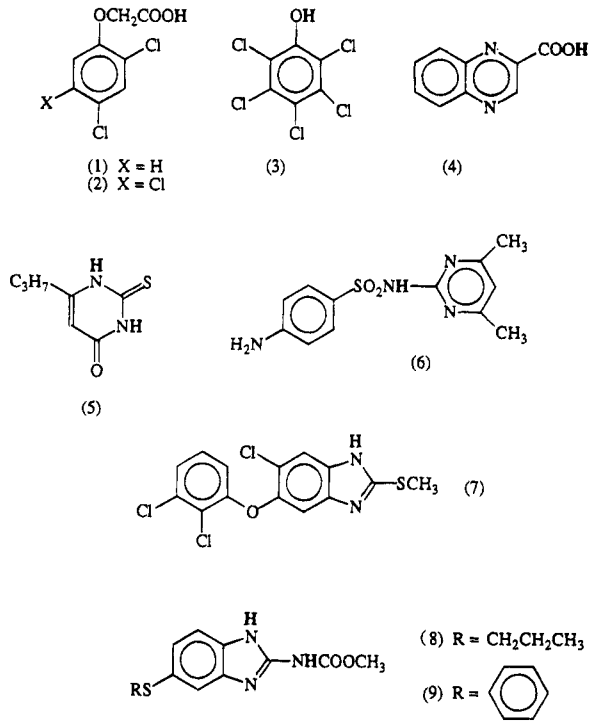

Figure 1. Structures of some acidic compounds derivatized by resin-mediated methylation in this work: **1**, 2,4-D; **2**, 2,4,5-T; **3**, pentachlorophenol; **4**, quinoxaline-2-carboxylic acid; **5**, 6-propyl-2-thiouracil; **6**, sulfadimidine; **7**, triclabendazole; **8**, albendazole; and **9**, fenbendazole. Methylation sites shown in boldface.

supercritical carbon dioxide as a reaction fluid for phase transfer methylations[7] and the high permeability of supercritical carbon dioxide encouraged us to examine the use of carbon dioxide in supercritical mode as the solvent in this resin-mediated methylation procedure.

We found the quaternary ammonium anion exchange resin to be a very effective support matrix for the methylation of strongly acidic organic analytes in supercritical carbon dioxide. The chlorophenoxyacetic acids **1** and **2** could be readily concentrated from a large volume of basic aqueous solution using AG MP-1 resin in the fluoride form and simply released as the methyl ester after reaction with methyl iodide in supercritical CO_2. Under the same conditions, this technique was found to be applicable to other strong monobasic acids such as pentachlorophenol (**3**) and quinoxaline-2-carboxylic acid (**4**, the marker compound used for the detection of residues of the antimicrobial compound carbadox). In comparison, yields of the methylation reaction were low when acetonitrile replaced supercritical CO_2 as the solvent.

In view of our previous success in derivatizing thiouracils with methyl iodide in acetonitrile,[9] the range of applicability of the SFE resin methylation procedure was tested on several other less acidic compounds including the 6-propyl-2-thiouracil (**5**), sulfadimidine (**6**), and the anthelmintics triclabendazole (**7**), fenbendazole (**8**) and albendazole (**9**). Structures of these compounds are shown in Figure 1.

In this initial paper, we describe the general methods and the parameters governing methylation by methyl iodide in supercritical carbon dioxide of analytes adsorbed onto ion exchange resins. The methylation yields obtained using this technique are compared to those obtained using liquid solvents such as acetonitrile.

EXPERIMENTAL SECTION

Standards and Reagents. AR grade toluene and HPLC grade methanol and acetonitrile were obtained from Mallinckrodt Australia (Clayton, Australia). Methyl iodide, AR grade, was supplied by Mallinckrodt Speciality Chemical Corp. (Paris, KY). For low-level determinations using the electron capture detector (ECD), methyl iodide was twice distilled in glass prior to use.

Pentachlorophenol, trichlorophenol, triclabendazole, albendazole, sulfadimidine, 2,4-D, 2,4,5-T, quinoxaline 2-carboxylic acid (QCA), 1,1-bis(4-fluorophenyl)-2,2-dichloroethene (difluoro-DDE), and 1,1-bis(4-bromophenyl)-2,2-dichloroethene (dibromo-DDE) were provided by the Curator of Standards, Australian Government Analytical Laboratories, Pymble, Australia. Diphenyl sulfone was supplied by Sigma. The methyl esters of 2,4-D and 2,4,5-T were prepared in this laboratory by methylation of 5 g of the appropriate acid with 20 mL of methanol containing 10% by weight concentrated sulfuric acid at 60 °C for 1 h, followed by cooling to 4 °C for 30 min and filtration under vacuum. The esters were twice recrystallized from hot methanol, followed by storage at 4 °C for 30 min before filtration.

Pentachloroanisole was prepared by vigorously shaking a mixture of 0.5 mL of dimethyl sulfate and 100 mg of pentachlorophenol dissolved in 0.1 M sodium hydroxide (20 mL) for 30 min and then filtering the crude product and recrystallizing twice from methanol. All solutions of the prepared methylated analytes gave a single peak by GC/MSD and had physical properties in accord with reported literature values.

A mixture of the two isomers of *N*-methyltriclabendazole (approximately 1:1) was prepared by stirring a mixture of triclabendazole (100 mg), anhydrous potassium carbonate (600 mg), methyl iodide (500 μL), and acetone (10 mL) overnight. After filtration and evaporation to dryness, the product, which consisted of a mixture of two monomethyl isomers by GC/MS, was used without further purification. It was free of triclabendazole when tested by TLC.

Resins and Support Material. The 200–400 mesh analytical grade AG1-X8 and AG MP-1 anion exchange resins were obtained from Bio-Rad Laboratories (Sydney, Australia) in the chloride form. The principal difference between these two strong quaternary ammonium resins is the pore diameter. Macroporous resins such as AG MP-1 are usually selected for use with organic solvents because even swelling of the resin in such solvents is small and leaves pores of sufficient size for analytes not to be excluded from the resin matrix. The resins were converted to their fluoride, hydroxide, cyanide, acetate, propionate, or butyrate form in a large glass chromatographic column using at least 20 bed volumes of a 1 M aqueous solution of the appropriate sodium salt. Apart from resins with a hydroxide counterion, bulk quantities of resins prepared in this way could be stored for several weeks without loss of activity after washing with water and then methanol and air-drying.

The use of AG MP-1 in the hydroxide, fluoride, acetate, propionate, or butyrate form did not significantly affect either the overall methylation yield or the type of products. In other work in this laboratory, we have found that the strongly basic hydroxide form of the resin caused rapid degradation of some base-sensitive compounds. By contrast, the fluoride form of the resin is essentially neutral and can be stored for prolonged periods without deterioration. Therefore, as with the thiouracil work,[9] this form was used routinely as the counterion for most of this work.

The best and most repeatable yields were obtained using the smallest bed volume resin bed examined (0.1 g), even for standard solutions containing up to 100 μg of analyte. The use of columns containing larger volumes of resin did not have a significant effect on product yield, provided that reagent quantities were also increased proportionately, so for reasons of economy, speed, and ease of processing, 0.1-g resin beds were preferred.

Preparation of Sample Solutions for Methylation and Extraction. The majority of the present exploratory work was carried out on the methylation and extraction of acidic analytes from standard solutions. Stock solutions at 0.5−1 mg/mL were prepared in acetonitrile. To dissolve some of the benzimidazoles, a 50% mixture of acetonitrile with 0.1 M aqueous NaOH was required. The sample to be derivatized was prepared by diluting an appropriate volume of stock solution (typically 100 μL) with 1−10 mL of 0.1 M sodium hydroxide.

Methylation Using Methyl Iodide in Supercritical CO_2. Prior to methylations with methyl iodide in supercritical carbon dioxide, analytes were adsorbed onto 0.1 g of dry ion exchange resin in a Pasteur pipet containing a glass wool plug. A solution of the acid to be derivatized (dissolved in aqueous 0.1 M sodium hydroxide) was vacuum aspirated through the resin bed. Three 1-mL aliquots of methanol were used to remove water from the resin before the resin was dried by aspirating air through it for 5 min. The dried resin was then transferred into one of the 2.5-mL Isco extraction vessels used throughout this work for SFE methylation. Neat methyl iodide (100 μL) was added to the top of the resin prior to pressurization with CO_2, and samples were allowed to react under pressure for 20 min with no flow. This reaction step was performed at a constant pressure of 200 bar and 80 °C and was followed by extraction of the products into toluene with 20 mL of pressurized CO_2 under the same conditions.

Methylation Using Methyl Iodide in Acetonitrile. Resin-mediated methylation using acetonitrile as the solvent was performed under ambient laboratory conditions on 0.1 g of dry AG MP-1 resin (fluoride form) weighed into a 3-mL polypropylene solid phase extraction (SPE) cartridge with a polyethylene frit. Analytes dissolved in 0.1 M aqueous sodium hydroxide were allowed to percolate through the cartridge under gravity. After removal of excess alkali by aspiration on a water pump, the resin was rinsed with three 1-mL portions of methanol and air-dried for 5 min on a vacuum manifold. The resin bed was saturated with 300 μL of a solution of 0.5 M methyl iodide in acetonitrile before the SPE tube was covered with aluminum foil and the methylation reaction was allowed to proceed for 1 h at room temperature. The reaction products were eluted with 1.5 mL of acetonitrile, and this solution was used for determinations by GC after addition of 0.5 mL of an internal standard solution.

Supercritical Fluid Extraction. An Isco Model SFX 2-10 extraction unit and an Isco Model 260D (Lincoln, NE) syringe pump were used throughout this work. Fused silica restrictor tubing of 50 mm i.d., approximately 25 cm in length, was used to control the flow rate to 0.9−1.5 mL/min of pressurized CO_2. Extractions were typically performed at a constant pressure of 200 bar. The quantity of pressurized CO_2 consumed was used to monitor the length of the extraction. This was the simplest way to overcome inconsistencies in flow rate. Extraction temperatures of 60−90 °C were used. Waste extraction fluid was vented through charcoal traps to minimize the release of methyl iodide and toluene.

Gas Chromatography. Analyses using the electron capture detector (ECD) were performed on a Hewlett-Packard Model 5890 GC. For analytes apart from phenols, separations were carried out using 1-μL splitless injections onto an HP Ultra 1 column (12 m × 0.2 mm i.d. × 0.33 μm film thickness). Helium (1 mL/min) was used as the carrier gas and nitrogen (50 mL/min) as the makeup gas. The injector temperature was held at 230 °C and the detector temperature at 300 °C. An initial oven temperature of 90 °C (70 °C for acetonitrile solutions) was held for 1 min and increased to 110 °C (90 °C for acetonitrile solutions) at 10 °C/min and then to 260 °C at 20 °C/min, where it was held for 7 min. For the analysis of methylated benzimidazoles, the temperature ramp was continued to 300 °C and then held for 5 min. For phenols, a 25-m HP Ultra 1 column (0.32 mm i.d., 0.52 μm film thickness) was used with temperature held at 130 °C for 3 min prior to ramping at 20 °C/min to 280 °C and holding 2 min.

GC/MS analyses were performed on a Hewlett-Packard 5890-(II) with a 5971 mass selective detector (MSD), under similar chromatographic conditions.

Quantitation by GC with Atomic Emission Detection (AED). Methyl derivatives for which authentic standards were unavailable were quantitated (after identification by GC/MS) using the compound-independent calibration capability of a Hewlett-Packard atomic emission detector (AED) coupled to a Hewlett-Packard 5890(II) GC. As the intensity of the signal from this detector is proportional to the number of atoms of a chosen element in the microwave plasma, the concentration of a given analyte of known atomic composition may be deduced by comparison of its signal to that of an internal standard of known concentration and molecular formula. GC conditions similar to those described above were employed with the AED. To reduce the possibility of inaccuracy resulting from chromatographic losses, it was preferred to use related compounds as references in this quantitation procedure. Sulfur-containing derivatives were quantitated using their sulfur emission against an internal standard of diphenyl sulfone or *N*-methyltriclabendazole. Other analytes were determined using the carbon channel of the AED by comparison to internal standards of diphenyl sulfone, dibromo-DDE, 2,4,5-T methyl ester, or *N*-methyltriclabendazole.

RESULTS AND DISCUSSION

The main aim of this work was to determine the viability of methylation on ion exchange resin and compare its efficiency with that of the phase transfer methylation technique we previously described[7] for the analysis of aqueous solutions containing 2,4-D and 2,4,5-T. Overall, resin-mediated methylation was found to be a robust procedure which tolerated a wide range of variables. Therefore, maximum methylation yields could be consistently obtained without any stringent requirement to operate under optimized conditions. As a consequence, the standard procedure described in the Experimental Section was developed primarily for convenience in routine sample handling.

Preliminary work showed that, as expected given the need to use nonaqueous solvents, the macroporous anion exchange resin AG MP-1 was more suitable than AG1-X8 for trapping and reacting analytes. The fluoride form of the resin was used in preference to the strongly basic hydroxide form, which is unstable and can cause rapid degradation of some base-sensitive compounds.

Resin-Mediated Methylation of Carboxylic Acids and Phenols in Supercritical Carbon Dioxide. Strong organic acids

Table 1. Yields of Methyl Derivatives of Acidic Compounds on AG-MP1 Anion Exchange Resin Using Methyl Iodide in Supercritical Carbon Dioxide

analyte	concn (ppm)	vol (mL)	replicates	yield (%)	CV (%)
2,4-D (**1**)	0.1	20	6	92	10
	0.005	200	1	94	
2,4,5-T (**2**)	0.035	20	6	99	11
	0.002	200	1	101	
PCP (**3**)	0.009	20	6	78	13
QCA (**4**)	10	1	5	87	10

Table 2. Influence of Quantity of Methyl Iodide Used during Resin-Mediated Methylation of Chlorophenoxyacetic Acids in Supercritical CO_2 Solvent

methyl iodide		yield methyl ester[c] (%)	
concn[a] (M)	vol[b] (μL)	2,4D	2,4,5-T
0.16	25	50	59
0.32	50	89	98
0.48	75	89	110
0.64	100	91	100
1.28	200	92	102

[a] Methyl iodide concentration in 2.5-mL SFE vessel after addition of CO_2. [b] Volume of neat methyl iodide used. [c] Single determination on 2 μg of 2,4-D and 0.7 μg of 2,4,5-T.

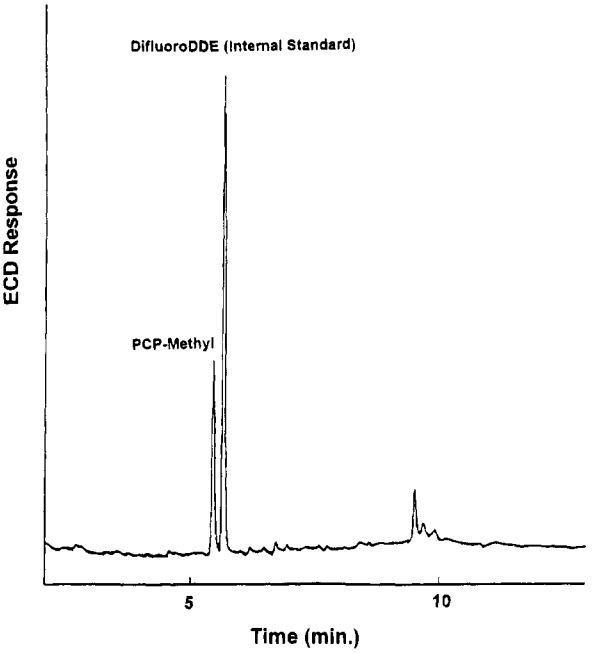

Figure 2. Resin-mediated methylation of pentachlorophenol extracted from 20 mL of tap water fortified at 9 ppb. GC/ECD chromatogram of extract following derivatization on AG MP-1 in supercritical CO_2.

such as carboxylic acids and halogenated phenols were efficiently trapped on anion exchange resins from both neutral and basic aqueous solutions. High yields were obtained for the methylation and subsequent release from the resin of these trial analytes using methyl iodide in supercritical CO_2 (see Table 1) when the procedure documented in the Experimental Section was used.

In line with our previous work on the use of supercritical CO_2 as a solvent in the phase transfer methylation of chlorophenoxyacetic acids, a pressure of 200 bar was used throughout this study. It was found that use of higher pressures had no significant effect on product yield. By contrast, raising the temperature was beneficial. For example, when derivatization was conducted at 50 °C, the yield of pentachloroanisole from pentachlorophenol was approximately half that obtained at 80 °C (78%). A minimum of 50 μL of methyl iodide was required to ensure complete methylation of chlorophenoxyacetic acids trapped on 0.1 g of resin (Table 2). Larger volumes of methyl iodide did not increase the yield. A minimum static reaction time of 8 min in the SFE cartridge was required to achieve the excellent derivatization yields shown in Table 1. Only 25–30% of the 2,4-D and 2,4,5-T in a sample was recovered if the reaction time was reduced to 2 min.

The yields of methyl esters from 20-mL aqueous samples fortified with 2,4-D and 2,4,5-T and methylated after adsorption onto AG MP-1 resin were excellent, as shown in Table 1, and similar to those obtained using the phase transfer methylation procedure[7] (90–100%). The volume of analyte solution used seemed to be unimportant, and the use of the ion exchange resin had two advantages over the phase transfer procedure. Whereas 3 mL was the largest volume of sample which could be processed using the phase transfer procedure, in this resin-mediated procedure, analytes could be concentrated from a large volume (200 mL) of solution onto a small quantity of resin (0.1 g) prior to derivatization and extraction into a small volume of solvent. As shown in Table 1, this led to no decrease in methylation efficiency and allowed much lower detection limits. In addition, extracts could also be injected directly into the GC without further cleanup. This derivatization technique was found to be simple, reliable, and repeatable and required minimal sample manipulation.

The ability to preconcentrate acidic analytes present at low concentration in aqueous samples is a particular advantage of this technique, which should be useful in environmental and trace organic residue analysis. The methylation of the common environmental contaminant pentachlorophenol (PCP) and QCA (a marker residue of the antimicrobial compound carbadox) was also examined to explore its potential in these areas. Both of these compounds were efficiently concentrated and methylated by the resin SFE method, as shown in Table 1. Figure 2 shows the ECD chromatogram obtained when 20 mL of water spiked at 9 ppb with PCP was analyzed. The mean recovery of six replicates at this level was 78%, with a 13% coefficient of variation. On the other hand, phenol and 4-nitrophenol, which are similar to but less acidic than PCP, gave no yield of methyl ethers. There was evidence in the chromatograms that phenol was eluted without derivatization under the SFE conditions used.

Resin-Mediated Methylation of Carboxylic Acids and Phenols in Acetonitrile. It seemed worthwhile to compare carbon dioxide and acetonitrile as reaction solvents for this procedure, as sample handling is even simpler when acetonitrile rather than supercritical CO_2 is used as the solvent, and batch processing of samples is possible. When acetonitrile was used, however, the yields of 2,4-D and 2,4,5-T methyl esters decreased to only 6–8% at room temperature (see Table 3). Treatment of the resin with a second portion of methyl iodide in acetonitrile released a further 6–8% of the methylated products. Similarly, when QCA, adsorbed onto macroporous resin, was subjected to derivatization with methyl iodide in acetonitrile at room temperature for 1 h, yields were less than 10%. Further methylated product was obtained by repeating the process up to nine times. To examine whether temperature was responsible for low yields

Table 3. Influence of Quantity of Methyl Iodide Used during Resin-Mediated Methylation of Sulfadimidine (100 μg) in an Acetonitrile Solvent

methyl iodide		
concn[a] (M)	vol[b] (μL)	derivative, yield (%)
0.1	2	45
0.25	5	55
0.5	10	65
1	20	64
1.5	30	66
2	40	65

[a] Methyl iodide concentration in 300 μL of acetonitrile solution. [b] Equivalent volume of neat methyl iodide. [c] Total of all methyl products (GC/AED quantitation). Mean of duplicate analyses.

of product, a methylation in acetonitrile was performed at 80 °C (with the reaction mixture sealed in an extraction cartridge in the SFE instrument) and gave yields of 45% in each of two sequential cycles of the methylation procedure (Table 3). Thus, although temperature has a definite effect on the methylation yield, supercritical CO_2 is clearly a particularly effective solvent for methylative displacement of organic acids from the resin. Other liquid solvents were no more effective than acetonitrile for this purpose; performing the reaction in either methanol or toluene resulted in yields of methyl QCA of approximately 5% in ambient temperature methylations. No pentachloroanisole was produced when resin-mediated methylation of PCP was attempted using an acetonitrile solvent.

Methylation of Organic Analytes Containing Acidic Nitrogens. The range of applicability of resin-mediated methylation in supercritical CO_2 was investigated using several other types of acidic compounds. These included analytes commonly determined using HPLC, for which GC confirmation techniques would be highly desirable. The acids tested included sulfadimidine (**6**), albendazole (**9**), triclabendazole (**7**), fenbendazole (**8**), and 6-propyl-2-thiouracil (**5**).

As these compounds are less acidic and less water soluble than the oxyacids discussed above, the parameters associated with adsorption onto the resin required more attention. The pH and ionic strength of sample solutions had a profound effect on the retention of analytes by the ion exchange resin. Although the hydroxide ion used to ensure alkalinity for efficient analyte trapping has a relatively low affinity for the resin, it was found to be necessary to avoid using it at concentrations higher than 0.1 M. Up to this concentration, yields of methyl derivatives of the benzimidazoles were essentially constant, but they rapidly deteriorated thereafter, showing a 30% yield reduction when 0.2 M NaOH was used. The recovery of methyltriclabendazole from the resin-mediated methylation technique dropped from 80% when 100 μg was loaded onto the resin at pH 13 to 40% at pH 10 and was barely detectable at pH 7 or lower.

Provided that pH and ionic strength were appropriately controlled, the volume of analyte solution used seemed to be unimportant. Samples containing 100 μg of albendazole gave a similar yield of the expected dimethylated products (80–90%) no matter whether their volume was 1 or 10 mL.

After the analytes were loaded in aqueous solution, it was essential to dry the resin using methanol and a stream of air prior to derivatization. This process was found to ensure maximum product yields, presumably as a result of ensuring access of the water-immiscible derivatizing agent to the analyte. Some compounds such as the benzimidazoles could be retained by hydrophobic interactions for immediate derivatization, even when presented to the resin as a solution in acetonitrile. However, the use of alkaline aqueous solutions resulted in the analytes being sufficiently strongly bound to the resin to be safely washed with methanol after sample loading without loss of underivatized analyte. It is envisaged that this washing step would also act as a cleanup to remove nonionic matrix interferences in real samples.

Table 3 shows the result of varying the concentration of methyl iodide in the derivatizing solution on the yield of methylated sulfadimidine. Changing the concentration of methyl iodide in acetonitrile from 0.5 to 2.0 M did not affect the methylation yield when reaction times of 30–60 min were used. Reaction times in the range 10–120 min had no apparent systematic effect on methylation yields for benzimidazoles and sulfadimidine; however, recoveries for reaction times less than 1 h seemed to be less reproducible.

The methylation of 6-propyl-2-thiouracil under SFE conditions with this method gave results which were very comparable to those obtained in the liquid phase using acetonitrile as the solvent (see Table 4). However, its total derivatization yield in super-

Table 4. Extraction and Methylation of Benzimidazoles, Sulfadimidine, and Propylthiouracil (100 μg Each) from AQ-MP1 Resin Using Methyl Iodide in Supercritical Carbon Dioxide or Acetonitrile

	acetonitrile			carbon dioxide		
		product[b]			product[b]	
analyte	yield[a] (%)	type	ratio	yield[a] (%)	type	ratio
albendazole (**5**)	85	dimethyl	5	50	dimethyl	5
		dimethyl	4		dimethyl	4
		monomethyl	4		monomethyl	4
fenbendazole (**8**)	80	dimethyl	8	50	dimethyl	6
		dimethyl	6		dimethyl	4
		monomethyl	1		monomethyl	5
triclabendazole (**7**)	80	monomethyl	3	50	monomethyl	3
		monomethyl	2		monomethyl	2
sulfadimidine (**6**)	65	monomethyl	40	15	monomethyl	1–5[c]
		dimethyl	1		dimethyl	1–5[c]
		trimethyl			trimethyl	5[c]
6-propyl-2	50	dimethyl	1	60	dimethyl	1
thiouracil (**5**)		dimethyl	15		dimethyl	15

[a] Sum of all methyl derivatives. [b] Degree of methyl substitution and approximate relative product abundance ratio. [c] Proportions highly variable.

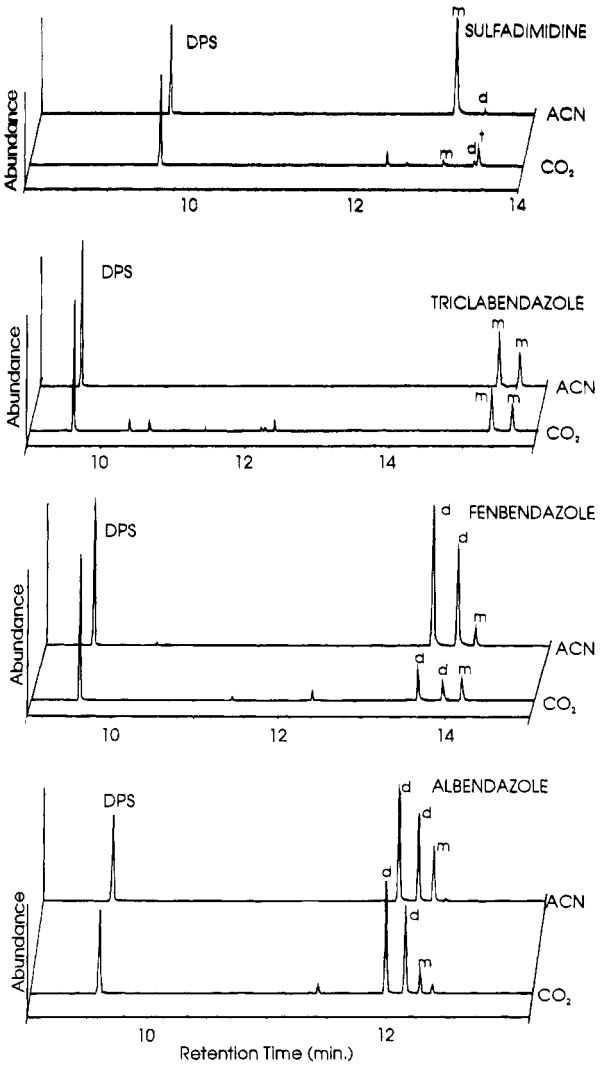

Figure 3. GC/MS chromatograms of methylated sulfadimidine and benzimidazoles following derivatization in either acetonitrile (ACN) or supercritical CO_2 of 100 μg trapped on AG MP-1 resin. Plots are offset slightly for clarity. DPS, diphenyl sulfone (internal standard). Monomethyl (m), dimethyl (d), and trimethyl (t) derivatives were identified by mass spectra.[10]

critical CO_2 was lower than that of the more acidic compounds discussed previously (see Table 1), as were those of sulfadimidine, triclabendazole, and the benzimidazoles (albendazole and fenbendazole). This group of compounds contains multiple acidic nitrogen atoms which may be methylated (as shown in Figure 1). The resin-mediated SFE methylation of these multifunctional analytes gave a smaller number of products (data in Table 4) than more aggressive procedures, such as on-column methylation using tetraalkylammonium salts.[10] Figure 3 shows chromatograms from the methyl iodide SFE derivatization of several benzimidazoles trapped on ion exchange resin. The degree of methylation of these products was determined by GC/MS with reference to previous assignments.[10] Albendazole and fenbendazole were each converted to a pair of isomeric dimethyl derivatives of approximately equal proportion and a monomethyl derivative. This result compares favorably with that obtained from on-column derivatization using nonselective trimethylphenylammonium (TMPA) salts[10] such as TMPA hydroxide or fluoride, which yielded significant proportions of up to five different methyl products[10] with a concomitant reduction in intensity of each. The esters produced in the current work were similar to those produced by milder on-column methylating agents such as TMPA in the cyanide form. Thus, although the usefulness of this technique for quantitative analysis of the benzimidazoles is limited by its generation of multiple products and by its relatively modest yields, the results obtained are no worse than those of some other methylation procedures. Thus, resin-mediated methylation is an easy and attractive alternative for the preparation of methylated derivatives for GC/MS confirmation of results following quantitative HPLC analysis.

The only compound in the classes discussed here which gave poor results under SFE conditions was sulfadimidine (see Table 4). A highly variable mixture of the mono-, di-, and trimethylated derivatives of sulfadimidine, with a total yield of approximately 15%, was obtained by SFE resin methylation. To test whether underivatized elution was responsible for the lower than expected yields obtained by SFE with the less acidic compounds, a second resin-mediated methylation was performed in acetonitrile on SFE extracts of sulfadimidine. After derivatization on the resin with methyl iodide in the normal way, the SFE extract was evaporated just to dryness and redissolved in 10% acetonitrile in 0.1 M aqueous NaOH and then passed through fresh AG MP-1 to trap any underivatized analyte. No evidence of methyl products was found when further methylation in acetonitrile was attempted on this resin sample, so reduced yields under SFE conditions may be due to either irreversible retention or decomposition on the resin.

It is noteworthy that sulfadimidine gave a very different product profile when reacted and extracted using supercritical CO_2 rather than acetonitrile as the solvent. The data in Table 4 indicate that the monomethyl derivative was by far the most abundant product in the latter system, with a minor quantity of the dimethyl derivative and no trimethyl. In contrast, although the proportion of each derivative was quite variable, trimethylated sulfadimidine was typically the most abundant product from resin-mediated methylation in supercritical CO_2. It seems possible that the low recovery of sulfadimidine from the SFE system is due to a poor solubility of the monomethyl derivative (which would be the most polar of the three derivatives) in supercritical CO_2, which is a much less polar solvent than acetonitrile.

Comparison of Supercritical Carbon Dioxide and Acetonitrile as Reaction Solvents. Supercritical CO_2 proved to be an excellent solvent for methylation of stronger organic acids such as 2,4-D and 2,4,5-T trapped on an ion exchange resin, giving very high yields of methyl esters and ethers (see Table 1). It also gave satisfactory yields for a wide range of the other analytes tested. In comparison, the data in Table 4 show that although acetonitrile had a slight yield advantage over supercritical CO_2 for the derivatization of benzimidazoles, the latter solvent was of more general applicability for resin-mediated methylation, giving methylated products in every instance. From this work at least, resin-mediated methylation in the SFE environment has greater practical utility because of its excellent concentration factors and the high yields obtained in analyses of strong acids isolated from aqueous solutions.

The acetonitrile methylation procedure was slightly more sparing on methyl iodide consumption. A minimum of 50 μL of methyl iodide was required to ensure complete methylation under

(10) Amijee, M.; Wells, R. J. *J. Chromatogr.* **1994**, *662*, 123–137.

SFE conditions of chlorophenoxyacetic acids trapped on 0.1 g of resin. By contrast, 300 μL of 0.5 M methyl iodide in acetonitrile (equivalent to approximately 10 μL of neat methyl iodide) was sufficient to maximize the yields of both sulfadimidine (see Table 3) and triclabendazole when acetonitrile was the solvent. With current technology, the acetonitrile procedure is more economical to perform, as it is extremely simple and is amenable to batch processing. However, the SFE procedure is simple and is more rapid for individual samples. The introduction of automated multisample SFE equipment will overcome disadvantages it may have in this area.

CONCLUSIONS

Resin-mediated methylation using methyl iodide in supercritical CO_2 as a reaction and elution medium is a simple and rugged method which allows large concentration factors for aqueous solutions of acidic analytes. The technique allows the derivatization and extraction of highly polar organic acids using SFE and is applicable to a broad range of acidic substances from benzimidazoles to carboxylic acids. It overcomes two of the limitations of phase transfer methylation with methyl iodide by allowing analysis of residues in large sample volumes and avoiding the need for subsequent removal of reagents before quantitation.

In acetonitrile, the recovery of strongly acidic substances such as chlorophenoxyacetic acid herbicides, pentachlorophenol, and quinoxaline-2-carboxylic acid from the resin as their methyl esters was incomplete and gave low yields. These compounds could only be methylated efficiently by this technique when supercritical carbon dioxide was used as the solvent. In acetonitrile, only the benzimidazoles, sulfadimidine, and thiouracils could be derivatized in satisfactory yield in a single step. The use of this solvent, however, requires unsophisticated equipment and small quantities of solvent and may be carried out in large batches with a good potential for automation.

This paper has described the general procedures for and range of applicability of resin-mediated methylation under different protocols. Specific methods for a wide variety of analytes based on these concepts have been developed and will be reported separately.

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