Journal of Chromatography, 629 (1993) 61–65
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 689

Optimization of sample application conditions for solid-phase extraction columns

Chang-Yuan L. Hsu and Rodney R. Walters

Drug Metabolism Research, The Upjohn Company, Kalamazoo, MI 49001 (USA)

ABSTRACT

When extraction recoveries during solid-phase extraction are affected by the volume or type of matrix applied to the column, the assay is not very robust. Using as examples the extraction of four basic drugs from biological matrices using cyano and octadecyl solid-phase extraction columns, it is shown that the recovery from 1 ml of plasma can be good while the recovery from water or diluted plasma is poor. Control of sample pH was found to increase recoveries from the cyano column by improving adsorption during sample application. Addition of detergent was found to enhance recoveries from the octadecyl column by moderating retention and allowing the drugs to be eluted more easily.

INTRODUCTION

Although solid-phase extraction (SPE) is widely used for the cleanup of biological samples prior to chromatographic analysis [1,2], developing rugged methods based on solid-phase extraction is still somewhat of an art. One problem that we have frequently encountered is a biofluid volume and/or type dependent recovery of drug and internal standard. There are three potential causes of poor recoveries: incomplete adsorption of drug to the SPE stationary phase during sample application with the 'lost' drug thus eluting in the initial flow-through of the column; premature elution during column-washing; or incomplete elution during the final elution step.

Experimentally determined differences in recoveries of drug from plasma, urine, or water must be due to chemical or physical differences between these matrices which affect either the properties of the solute or the stationary phase. The most likely factors causing matrix-dependent recoveries are pH, ionic strength, and surface tension. This presentation will describe the results of experiments aimed at identifying the crucial variables for assay methods for two basic compounds extracted using cyano SPE columns and two other basic compounds extracted using C_{18} SPE columns, and show how the assays could be made more rugged with respect to recovery from water, plasma, and urine samples of various volumes.

Correspondence to: R. R. Walters, Drug Metabolism Research, The Upjohn Company, Kalamazoo, MI 49001, USA.

EXPERIMENTAL

Materials

The compounds tested for recovery, U-77567, U-88055, U-70226, and U-74747, were obtained from Upjohn (Kalamazoo, MI, USA). Sequanal quality trifluoracetic acid (TFA) was from Pierce (Rockford, IL, USA). Other chemicals were reagent grade and solvents were of HPLC or UV grade.

Apparatus

Solid-phase extraction was performed using Bond-Elut C_{18} and Cyano SPE columns containing 100 mg of stationary phase and a Vac-Elut solid-phase extraction manifold (Analytichem, Harbor City, CA, USA). The HPLC system used for the

measurement of U-77567 and U-88055 recoveries consisted of a Beckman 110B solvent delivery module set at a flow-rate of 1 ml/min, a Perkin-Elmer ISS-100 autosampler with a temperature-controlled sample tray thermostatted to 2°C, a Zorbax RX C_8 analytical column (5 μm, 250 × 4.6 mm I.D.) with a guard column (12.5 × 4.0 mm I.D.), and a Waters 470 fluorescence detector with an excitation wavelength of 275 nm and an emission wavelength of 310 nm. Samples were injected every 15 min and eluted isocratically with a mobile phase consisting of acetonitrile–0.01 *M* sodium acetate, pH 4.0 (10:90, v/v). The HPLC system used for the assay of U-70226 and U-74747 was a complex column-switching reversed-phase system which will be described separately [3].

Procedure

A 50-μl volume of a 200-ng/ml aqueous solution of U-77567 was mixed with 50 μl of a 300-ng/ml aqueous solution of U-88055 and various volumes of buffer, detergent, plasma (rat), urine (rat), etc. as shown in Table I. Additional water was added to bring the volume of each sample to at least 1 ml. The mixtures were applied to Bond-Elut Cyano SPE columns which had been preconditioned with 1 ml each of methanol and water. Following sample application, the columns were washed with 2 ml of water, dried for 3 min by sucking air through the columns using house vacuum (*ca.* 70 kPa), washed with 300 μl of hexane, and eluted by gravity with 300 μl of methanol containing 0.1% TFA. The last volume was forced out using pressurized nitrogen. Following evaporation and reconstitution, the recoveries were measured by reversed-phase HPLC against a non-extracted solution of the compounds.

A 10-μl volume of an acetonitrile solution containing 1100 ng/ml of U-70226 and 1500 ng/ml of U-74747 was mixed, in duplicates, with various solutions (including human plasma and urine) as shown in Table II, then applied to Bond-Elut C_{18} SPE columns which had been preconditioned with 1 ml each of methanol and water. Following sample application, the columns were washed with 1 ml of acetonitrile–methanol–water (25:25:50), dried for 3 min by sucking air through the columns using house vacuum (*ca.* 70 kPa), washed with 300 μl of hexane, dried again in the same manner for 10 min, and eluted by gravity with 500 μl of acetone–acetonitrile–triethylamine (TEA) (50:50:0.2). The last volume was forced out of the column using pressurized nitrogen. After evaporation, the samples were

TABLE I

RECOVERIES OF U-77567 AND U-88055 FROM VARIOUS MATRICES AFTER EXTRACTION USING CYANO SPE COLUMNS

Treatment		U-77567 recovery (%)	U-88055 recovery (%)
Water	1 ml	2	2
Plasma	0.01 ml	29	29
	0.1 ml	95	99
	1 ml	100	106
Urine	0.01 ml	16	16
	0.1 ml	96	99
	1 ml	103	109
0.1 *M* pH 5 buffer	0.1 ml	57	53
	1 ml	70	81
0.1 *M* pH 7 buffer	0.1 ml	101	105
	1 ml	101	105
0.1 *M* pH 9 buffer	0.1 ml	96	99
	1 ml	99	104
Saline	0.1 ml	34	30
	1 ml	37	43
5% Dextrose	0.1 ml	3	2
	1 ml	3	2
0.04% Tween 80	1 ml	17	2
0.04% Triton X-100	1 ml	4	4

TABLE II

RECOVERIES OF U-70226 AND U-74747 FROM VARIOUS MATRICES AFTER EXTRACTION USING C_{18} SPE COLUMNS

Treatment		U-70226 Recovery (% ± S.D.) (n = 2)	U-74747 Recovery (% ± S.D.) (n = 2)
Plasma + 0.05 *M* pH 7 buffer–0.1% TEA	1 ml each	106 ± 1	104 ± 3
Urine + 0.05 *M* pH 7 buffer–0.1% TEA	0.1 ml urine, 1 ml buffer	76 ± 13	51 ± 10
Water + 0.05 *M* pH 7 buffer–0.1% TEA	1 ml each	78 ± 0	54 ± 3
Water	1 ml	54 ± 1	33 ± 1
Plasma	1 ml	71 ± 3	68 ± 3
Plasma	0.1 ml + 0.9 ml water	67 ± 2	59 ± 1
Urine	1 ml	66 ± 1	46 ± 1
Urine	0.1 ml + 0.9 ml water	59 ± 4	40 ± 5
0.1 *M* pH 5 buffer	1 ml	64 ± 0	48 ± 1
0.1 *M* pH 7 buffer	1 ml	55 ± 2	41 ± 2
0.1 *M* pH 9 buffer	1 ml	79 ± 1	60 ± 0
Saline	1 ml	73 ± 3	53 ± 1
Saline–0.1% TEA	1 ml	29 ± 6	42 ± 7
5% Dextrose	1 ml	57 ± 6	36 ± 6
0.04% Tween 80	1 ml	96 ± 3	104 ± 1
0.04% Triton X-100	1 ml	94 ± 1	95 ± 2
0.04% Triton X-100–0.1% TEA	1 ml	57 ± 2	97 ± 2

derivatized with 1-naphthyl isocyanate [4] and assayed by reversed-phase column-switching HPLC [3]. Recoveries were measured against a derivatized, non-extracted solution of the compounds.

The buffers used to dilute the samples in Tables I and II were all sodium phosphate buffers of the indicated pH and molarity. The pH of each buffer containing TEA was adjusted after addition of TEA.

RESULTS AND DISCUSSION

U-77567 (Fig. 1) is a basic compound assayed in biofluids using solid-phase extraction (cyano column) followed by reversed-phase HPLC. Recoveries of drug and internal standard (U-88055, Fig. 1) were high for 0.1–1 ml samples of both plasma and urine but low if 10 μl of plasma or urine were diluted to 1 ml with water prior to extraction due to their high drug content (same total amount of drug in all cases). It was suspected that this biofluid volume-dependent extraction recovery problem was caused by incomplete adsorption of drug to the cyano phase during sample application. Therefore, several experimental factors, such as pH, ionic strength, and the use of detergents, were tested for their effects on drug recovery.

The data from the experiments with U-77567 and U-88055 are shown in Table I. The results clearly

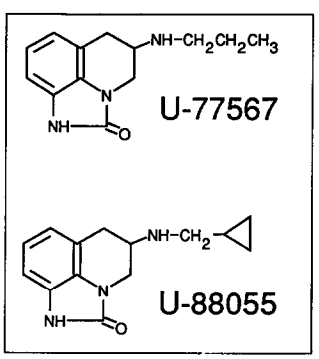

Fig. 1. Structures of U-77567 and U-88055, which were solid-phase extracted using a cyano column.

show the diminished recoveries when water or small plasma or urine volumes were extracted. This was obviously a pH effect, since addition of pH 7 or 9 buffers to the samples resulted in high recoveries, whereas ionic strength changes and addition of detergents did not alter the recoveries substantially. Since the pK_a values of these compounds are approximately 8, it can be concluded that the recovery problem was a simple equilibrium problem, with retention being too weak when the pH was not optimal. Apparently large plasma and urine samples provided their own buffering capacity, while samples diluted with water did not have sufficient buffering capacity to control the pH of the column.

U-70226 (Fig. 2), another basic drug, and its internal standard (U-74747, Fig. 2) were assayed by reversed-phase HPLC after solid-phase extraction on an octadecyl phase and derivatization of the alcohol moiety with 1-naphthyl isocyanate [3,4]. It was discovered that the recovery of drug from plasma samples was greater than from water and from other biofluids including urine, tissue homogenates, saliva and microsome samples. This problem was initially believed to be a result of variable adsorption during application to the SPE column. An experiment was conducted in which the compounds were applied to the SPE column in 1 ml of water. The flow-through was collected, mixed with 1 ml of plasma and 1 ml of pH 7 buffer–0.1% TEA, and reextracted (the latter conditions were known to result in high recoveries, see Table II). However, the compounds were not found in the initial flow-through, indicating that the problem was not one of poor initial adsorption.

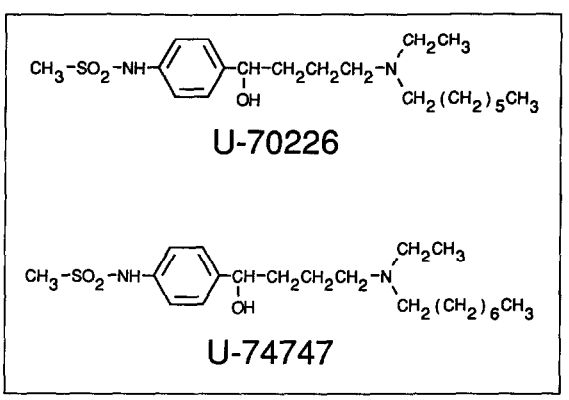

Fig. 2. Structures of U-70226 and U-74747, which were solid-phase extracted using an octadecyl column.

The original SPE column was then eluted three times with 0.5 ml volumes of the acetone–acetonitrile–TEA elution solvent. The recoveries of U-70226 and U-74747 were 67 ± 7% and 48 ± 7%, respectively, in the first fraction, 14 ± 3% and 11 ± 2% in the second fraction, and none in the third fraction. The total recoveries of 81% and 59%, respectively, after elution along with the lack of drug in the application flow-through suggest that these compounds are tightly adsorbed to the SPE stationary phase and are incompletely eluted by the elution solvent. In contrast, when the sample was applied in plasma, quantitative recoveries were achieved in the first 0.5 ml elution volume (Table II).

This problem was further examined by applying the compounds in a variety of solutions (Table II). Recoveries were lower when the drugs were mixed with water than when mixed with urine, and lower in urine than in plasma. Smaller volumes of plasma or urine (with additional water added) also resulted in lower recoveries. Recoveries were not improved by mixing the drugs with buffers of various pH values, saline, or dextrose. Addition of detergents, either Triton X-100 or Tween 80, did result in nearly quantitative recoveries. Addition of a pH 7 buffer containing TEA to the drugs in urine, water or plasma also improved recoveries, with recoveries being quantitative in the latter case. However, unbuffered TEA solutions (in saline or detergent) resulted in lower recoveries.

These data suggest that the C_{18} SPE phase contains strong adsorption sites. Some of these may be strong hydrophobic sites, others may be polar sites from exposed silanol groups. Addition of plasma, detergents, or buffered TEA during sample application each help to mask some of these sites, resulting in higher elution recoveries of drug. The highest recoveries were obtained by applying the samples in plasma and buffered TEA or in a detergent solution.

CONCLUSIONS

In the two examples given, matrix type and volume-dependent recoveries were found to be improved by addition of various reagents to the sam-

ple solution applied to the SPE columns. Testing the effects of buffers, detergents, etc. on solid-phase extraction recoveries during method development is useful in designing more rugged assays. If critical parameters can be identified, it may be possible to design assays such that any volume or type of matrix can be assayed without modification of the assay procedure.

REFERENCES

1 *Applications Bibliography Sample Preparation Products*, Varian Sample Preparation Products, Harbor City, CA 1991.
2 P.D. MacDonald (Editor), *Waters Sep-Pak Cartridge Application Bibliography*, Waters, Milford, MA, 5th ed., 1991.
3 C.L. Hsu and R.R. Walters, in preparation.
4 C.L. Hsu and R.R. Walters, *J. Chromatogr.*, 550 (1991) 621.