SHIMADZU

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Advanced sample preparation in LC/MS bioanalysis using new solid phase extraction

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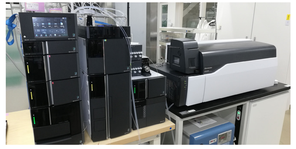
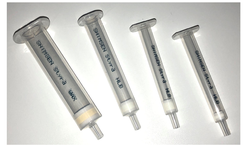
1. Introduction

・Sample preparation is a necessary step for LC/MS analysis. The sample preparation significantly impacts throughput, data quality and analytical cost. Selection and optimization of the method is essential for successful method development.

・In bioanalysis, solid phase extraction (SPE) is chosen as one of the best options for sample preparation to extract analyte from complex samples such as blood.

・We developed new SPE method of typical pharmaceutical compounds for biological sample. Recovery of compounds using new SPE was greater than 80% with a relative standard deviation of less than 7%. The method saves processing time without sacrificing recovery of target compounds.





LC-ESI-MS system for target pharmaceuticals quantitation and SPE cartridge SHIMSEN Styra series.

2. Materials and Methods

Sample preparation for spiked 10 compounds (Figure 1) in human plasma was carried out by new SPE method with SHIMSEN Styra HLB 30mg/1mL (P/N: 380-00855-09, Shimadzu (Shanghai) Global Laboratory Consumables Co., Ltd.). SPE pretreatment of plasma samples used the protocol shown in Figure 2. Treated samples were measured using a Nexera X3 UHPLC system and LCMS-8050 triple quadrupole mass spectrometer with MRM mode (Shimadzu Corporation). The target compounds were eluted from a Shim-pack Scepter C18-120 (Shimadzu Corporation) with a gradient of acetonitrile, and compounds were detected in positive mode ESI (Table 1).

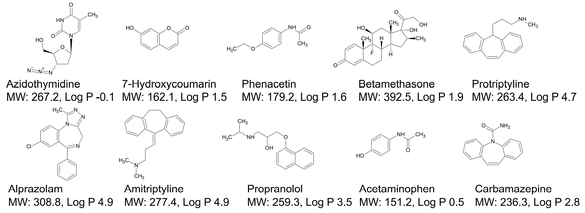


Figure 1 Chemical structures and its Log P value of target pharmaceuticals.

	Procedure		Volume	Flow rate
Step 1	Conditioning	MeOH	1mL	one drop/sec
Step 2	Equilibrate	Water	1mL	
Step 3	Sample load	Spiked plasma 200µL+4% H3PO4 200µL Spiked plasma (10µL Standard mix* +240 µL plasma)	400µL	
Step 4	Wash	5%MeOH	400µL	
Step 5	Elute	MeOH	500µL	

*The concentration of Standard mix is shown in the text.

Figure 2 Protocol for plasma sample pre-treatment by SHIMSEN Styra HLB 30mg/1mL (P/N: 380-00855-09).

Table 1 Analytical conditions of LC-ESI-MS.

LC
Column : Shim-pack Scepter C18-120 (150 mm L. x 2.1 mmI.D., 1.9 um, P/N: 227-30947-05)
Mobile phase : A 0.1 % formic acid in water, B 0.1% formic acid in acetonitrile
Time program : 20%B(0-0.3 min)-30%B(2 min, curve 2)-90%B(5 min, curve 6)-20%B(6 min)-STOP
Flow rate : 0.5 mL/min
Column temp. : 40℃
injection vol. : 1 µL

ESI-MS
Ionization mode : ESI positive
Probe voltage : +4.5kV
Interface temp. : 300℃
DL temp. : 250℃
Heat block temp. : 400℃
Nebulizing gas flow : 3 L/min
Drying gas flow : 10 L/min
Heating gas flow : 10L min
Mode : MRM

Compound Name	Polarity	MRM Transition m/z
Azidothymidine (AZT)	+	268.1 > 127.1
7-Hydroxycoumarin (7HC)	+	163.1 > 107.1
Phenacetin (Phen)	+	180.1 > 110.1
Betamethasone (BetaMeth)	+	393.1 > 373.1
Protriptyline (Protrip)	+	264.1 > 155.1
Alprazolam (Alplaz)	+	309.1 > 281.1
Amitriptyline (Amitrp)	+	278.1 > 91.1
Propranolol (Propra)	+	260.1 > 116.1
Acetaminophen (AcA)	+	152.1 > 110.1
Carbamazepine (CAR)	+	237.1 > 194.1

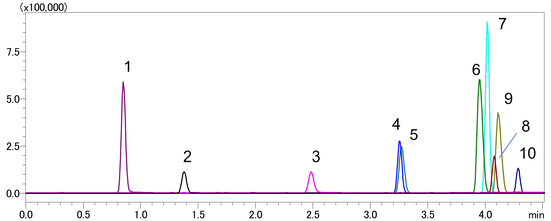


Figure 3 MS chromatogram of standard mixture of 10 target pharmaceuticals.

1. Acetaminophen: 160 ng/mL, 2. Azidothymidine: 160 ng/mL, 3. 7-Hydroxycoumarin: 160 ng/mL, 4. Phenacetin: 16 ng/mL, 5. Propranolol: 16 ng/mL, 6. Protriptyline: 16 ng/mL, 7. Carbamazepine: 16 ng/mL, 8. Betamethasone: 160 ng/mL, 9. Amitriptyline: 16 ng/mL, 10. Alprazolam: 16 ng/mL.

3. Results

3-1. Comparison of recoveries in real samples by SPE

Figure 3 shows MS chromatogram of a mixture of pharmaceutical compounds under the LC/MS conditions used for this evaluation. All compounds were eluted within 5 minutes.

Using SHIMSEN Styra HLB and five commercially available products from several manufacturers, we processed plasma containing compound mixtures, and obtained its recovery rates. The concentration of AZT, 7HC, BetaMeth and AcA in the standard mixture is 10ug/ml in 40% acetonitrile, and the concentration of Phen, Protrip, Alpraz, Amitrp, Propra and CAR is 1ug/ml. When plasma-spiked drug mixtures were added to the respective manufacturer's SPEs according to the protocol in Figure 2, two products failed to pass the sample, even at a pressure of 560 mmHg (maximum manifold pressure). It is believed the viscous plasma did not pass through due to the high density of the packing materials in these product.

Table 2 Recovery of compounds for each SPE product.

Product	AZT	7HC	Phen	BetaMeth	Protrip	Alplaz	Amitrip	Propra	AcA	CAR
SHIMSEN 1	108%	103%	94%	105%	96%	112%	101%	109%	155%	97%
SHIMSEN 2	110%	111%	99%	112%	97%	121%	100%	108%	153%	101%
SHIMSEN 3	115%	112%	100%	110%	97%	114%	98%	107%	153%	104%
SHIMSEN 4	113%	111%	100%	119%	96%	124%	97%	108%	156%	106%
SHIMSEN 5	108%	111%	92%	109%	87%	112%	91%	97%	146%	101%
A-1	108%	113%	80%	121%	76%	138%	85%	87%	132%	105%
A-2	110%	115%	87%	130%	80%	148%	87%	91%	135%	112%
A-3	111%	122%	85%	134%	78%	155%	90%	91%	142%	112%
B-1	89%	76%	57%	139%	41%	164%	51%	56%	141%	60%
B-2	95%	78%	60%	135%	46%	160%	57%	62%	142%	66%
B-3	94%	76%	60%	136%	45%	164%	58%	62%	148%	65%
C-1	110%	111%	92%	126%	77%	136%	83%	86%	143%	104%
C-2	104%	113%	89%	120%	74%	132%	79%	84%	134%	105%
C-3	115%	122%	96%	128%	74%	147%	85%	88%	146%	106%

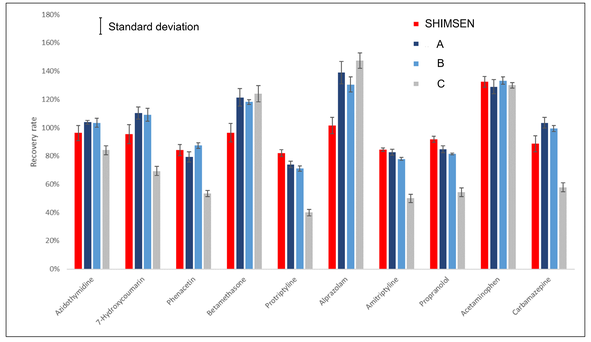


Figure 4 Compound recovery rate after SPE treatment by each product.

Table 3 Relative standard deviation of recovery for each compound.

Product	AZT	7HC	Phen	BetaMeth	Protrip	Alplaz	Amitrip	Propra	AcA	CAR
SHIMSEN	5.4%	7.0%	4.9%	6.8%	3.0%	5.6%	1.4%	2.3%	2.9%	6.5%
A	1.3%	3.9%	4.5%	5.1%	3.2%	5.7%	2.4%	2.9%	3.9%	3.7%
B	3.9%	4.6%	4.0%	4.7%	5.6%	3.7%	5.9%	5.5%	1.5%	5.6%
C	3.2%	4.2%	2.3%	1.4%	2.5%	4.0%	1.4%	0.7%	2.0%	2.1%

The percent recovery was obtained by the ratio of the peak area of extracted sample with spiked compounds to the peak area of standard compounds. The average recovery of new SPE was greater than 80% and standard deviation was less than 7%. This result was similar to that of conventional SPE.

3-2. Comparison of solvent elution time as usability

Every step (preconditioning, loading, washing and eluting) within a SPE procedure is imperative, and normally, it is necessary to pass the solvent forcibly using aspiration or pressurization. The new SPE cartridge has high flow rate and does not require aspiration or pressurization. Solvent elution times were compared between new SPE cartridge and 5 commercially available SPEs using 200 µL methanol under natural fall conditions. The new SPE method performed just 34 sec. In contrast, it took 61~150 sec to elute solvent and large standard deviation using conventional SPEs.

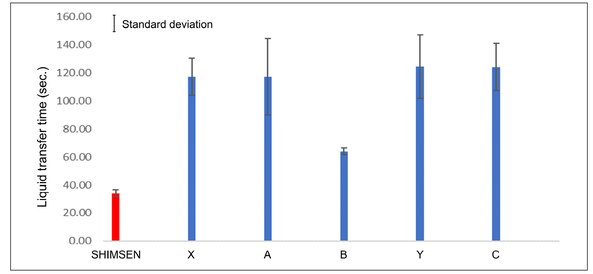


Figure 5 Liquid transfer time for each SPE product.

4. Conclusions

- The Recovery rate for SHIMSEN styra HLB are grater than 80% and %RSD are less than 7%. They are good and equivalent to other manufacturer's specification.
- The new SPE method enables to reduce processing time without aspirator or pressure device with high recovery of compounds in plasma.