BHIMADZU

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

Toshikazu Minohata¹; Ai Tsutsui¹; Haijuan An²; Keiko Shiren¹; Satoshi Yamaki¹ ¹SHIMADZU Corporation, Kyoto, Japan; ²Shimadzu (Shanghai) Global Laboratory Consumables Co., Ltd., Beijing, China

. 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	\ \	/olume	Flow rate	3. Res	ults									
Step 1	Conditioning	MeOH		1mL												
Step 2	Equilibrate	Water		1mL		3-1. CO	mpar	ISON	ot re	coveri	es in	real	sam	JIES I	JY 3F	Έ
		Spiked plasma 2	200µL+4%			Figure 3	shows	MS chr	omatog	gram of a	mixture	of pha	rmaceu	tical co	mpound	ds
		H3PO4 200µL				under the	LC/MS	conditic	ons use	d for this	evaluat	ion. Al	l compo	unds w	vere elu [.]	ted
Step 3	Sample load	Spiked plasma ((10µL	400µL	one drop/sec	within 5 m	nutes									
		Standard mix* +	240 µL			Lleina St		1 Styra	HIR or	nd five co	mmorci		ailahla n	roducto	from	
		plasma)				Using Si		N Olyra				ally ave				I
Step 4	Wash	5%MeOH		400µL		several ma	anutacti	lrers, w	e proce	essed pla	sma co	ntainin	g compo	buna mi	xtures,	and
Step 5	Elute	MeOH		500µL		obtained it	s recov	ery rate	es. The	concentra	ation of	AZT, 7	HC, Bet	aMetha	and AcA	A in
ŕ	*The concentration	on of Standard mix is	shown in the text			the standa	rd mixtu	ure is 10	Oug/ml	in 40% a	cetonitri	le, and	the cor	ncentrat	ion of F	Phen,
						Protrip, Alp	oraz, An	nitrp, Pr	opra a	nd CAR is	s 1ug/m	I. Whe	n plasm	a-spike	d drug	
Figure		or plasma sample	pre-treatment	by Shiivi	SEN Styra HLB	mixtures w	vere ado	ded to th	he resp	ective ma	anufacti	ırer's S	PEs ac	cordina	to the	
30mg/1	1mL (P/N: 380)-00855-09).				nrotocol in	Figure	$2 t_{\rm MO}$	nroduct	te failed to	n nace t	ho sam		n at a	nraccur	o of
									product				the vie			d not
Table 1	Analytical cor	nditions of I C-FS	SI-MS) (maxir	num ma		pressure). It is de	elleved		cous pla	asma di	α ποι
LC						pass thou	gn uue		light de	insity of th	ε μαυκι	ny mai		และระ	product	•
Column	· Shim-pack	Scepter C18-120 (15)	0 mm L, x 2,1 mm	I.D., 1.9 um),	Tahla 2 R		v of cor	nnounc	le for pac						
	P/N· 227-3	0.00000000000000000000000000000000000					recover	y UI CUI	iipound	13 101 646	II SFE þ	product	•			
Mobile pha	P/N: 227-3 ase : A 0.1 % for	0947-05) mic acid in water, B 0	0.1% formic acid in	n acetonitrile		Product	AZT	7HC	Phen	BetaMeth	Protrip	oroduct Alplaz	Amitrip	Propra	AcA	CAR
Mobile pha Time prog	P/N: 227-3 ase : A 0.1 % for ram : 20%B(0-0	0947-05) mic acid in water, B 0 .3 min)-30%B(2 min,	0.1% formic acid in curve 2)-90%B(5	n acetonitrile min, curve 6	e 6)-20%B(6 min)-STOP	Product SHIMSEN 1	AZT 108%	7HC 103%	Phen 94%	BetaMeth 105%	Protrip 96%	Droduct Alplaz 112%	Amitrip 101%	Propra 109%	AcA 155%	CAR 97%
Mobile pha Time progr Flow rate Column te	P/N: 227-3 ase : A 0.1 % for ram : 20%B(0-0 : 0.5 mL/min emp. : 40°C	0947-05) mic acid in water, B 0 .3 min)-30%B(2 min,	0.1% formic acid in curve 2)-90%B(5	n acetonitrile min, curve 6	e 5)-20%B(6 min)-STOP	Product SHIMSEN 1 SHIMSEN 2	AZT 108% 110%	7HC 103% 111%	Phen 94% 99%	BetaMeth 105% 112%	Protrip 96% 97%	Alplaz 112% 121%	Amitrip 101% 100%	Propra 109% 108%	AcA 155% 153%	CAR 97% 101%
Mobile pha Time prog Flow rate Column te injection ve	P/N: 227-3 ase : A 0.1 % for ram : 20%B(0-0 : 0.5 mL/min emp. : 40°C ol. : 1 μL	0947-05) mic acid in water, B 0 .3 min)-30%B(2 min,	0.1% formic acid in curve 2)-90%B(5	n acetonitrile min, curve 6	e 5)-20%B(6 min)-STOP	Product SHIMSEN 1 SHIMSEN 2 SHIMSEN 3	AZT 108% 110% 115%	7HC 103% 111% 112%	Phen 94% 99% 100%	BetaMeth 105% 112% 110%	Protrip 96% 97% 97%	Alplaz 112% 121% 114%	Amitrip 101% 100% 98%	Propra 109% 108% 107%	AcA 155% 153% 153%	CAR 97% 101% 104%
Mobile pha Time progr Flow rate Column te injection ve	P/N: 227-3 ase : A 0.1 % for ram : 20%B(0-0 : 0.5 mL/min emp. : 40°C ol. : 1 μL	0947-05) mic acid in water, B 0 .3 min)-30%B(2 min, 4 	0.1% formic acid in curve 2)-90%B(5	n acetonitrile min, curve 6 Polarity	6)-20%B(6 min)-STOP	Product SHIMSEN 1 SHIMSEN 2 SHIMSEN 3 SHIMSEN 4	AZT 108% 110% 115% 113%	7HC 103% 111% 112% 111%	Phen 94% 99% 100%	BetaMeth 105% 112% 110% 119%	Protrip 96% 97% 96%	Alplaz 112% 121% 114% 124%	Amitrip 101% 100% 98% 97%	Propra 109% 108% 107% 108%	AcA 155% 153% 153% 156%	CAR 97% 101% 104% 106%
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Mobile pha Time progr Flow rate Column te injection version SI-MS Ionization r Probe volta	P/N: 227-3 ase : A 0.1 % for ram : 20%B(0-0 : 0.5 mL/min emp. : 40°C ol. : 1 μL mode : ESI p age : +4.5k emp. : 300°C	0947-05) mic acid in water, B 0 .3 min)-30%B(2 min, Contraction of the second s	0.1% formic acid in curve 2)-90%B(5 mpound Name /midine (AZT) xycoumarin (7HC) etin (Phen)	n acetonitrile min, curve 6 Polarity + + + +	5)-20%B(6 min)-STOP MRM Transition <u>m/z</u> 268.1 > 127.1 163.1 > 107.1 180.1 > 110.1 303.1 > 373.1	Product SHIMSEN 1 SHIMSEN 2 SHIMSEN 3 SHIMSEN 4 SHIMSEN 5 A-1 A-2	AZT 108% 110% 115% 113% 108% 108% 108% 108%	7HC 103% 111% 111% 111% 111% 111% 111% 111% 111% 111% 111% 111% 111% 113% 115% 1020%	Phen 94% 99% 100% 100% 92% 80% 80% 87%	BetaMeth 105% 112% 110% 110% 110% 110% 110% 110% 110% 110% 110% 110% 110% 110% 121% 121% 1240/	Protrip 96% 97% 97% 97% 96% 97% 980% 78%	Alplaz 112% 121% 124% 124% 138% 148% 1550/	Amitrip 101% 100% 98% 97% 97% 91% 85% 85%	Propra 109% 108% 107% 108% 97% 87% 91%	AcA 155% 153% 153% 156% 146% 132% 135%	CAR 97% 101% 104% 106% 106% 105% 112%
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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.





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



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.



ole 3 Relative standard deviation of recovery for each compound.												
Product	AZT	7HC	Phen	BetaMeth	Protrip	Alplaz	Amitrip	Propra	AcA	CAR		
HIMSEN	5.4%	7.0%	4.9%	6.8%	3.0%	5.6%	1.4%	2.3%	2.9%	6.5%		
Α	1.3%	3.9%	4.5%	5.1%	3.2%	5.7%	2.4%	2.9%	3.9%	3.7%		
В	3.9%	4.6%	4.0%	4.7%	5.6%	3.7%	5.9%	5.5%	1.5%	5.6%		
С	3.2%	4.2%	2.3%	1.4%	2.5%	4.0%	1.4%	0.7%	2.0%	2.1%		

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 μ L 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.





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.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.