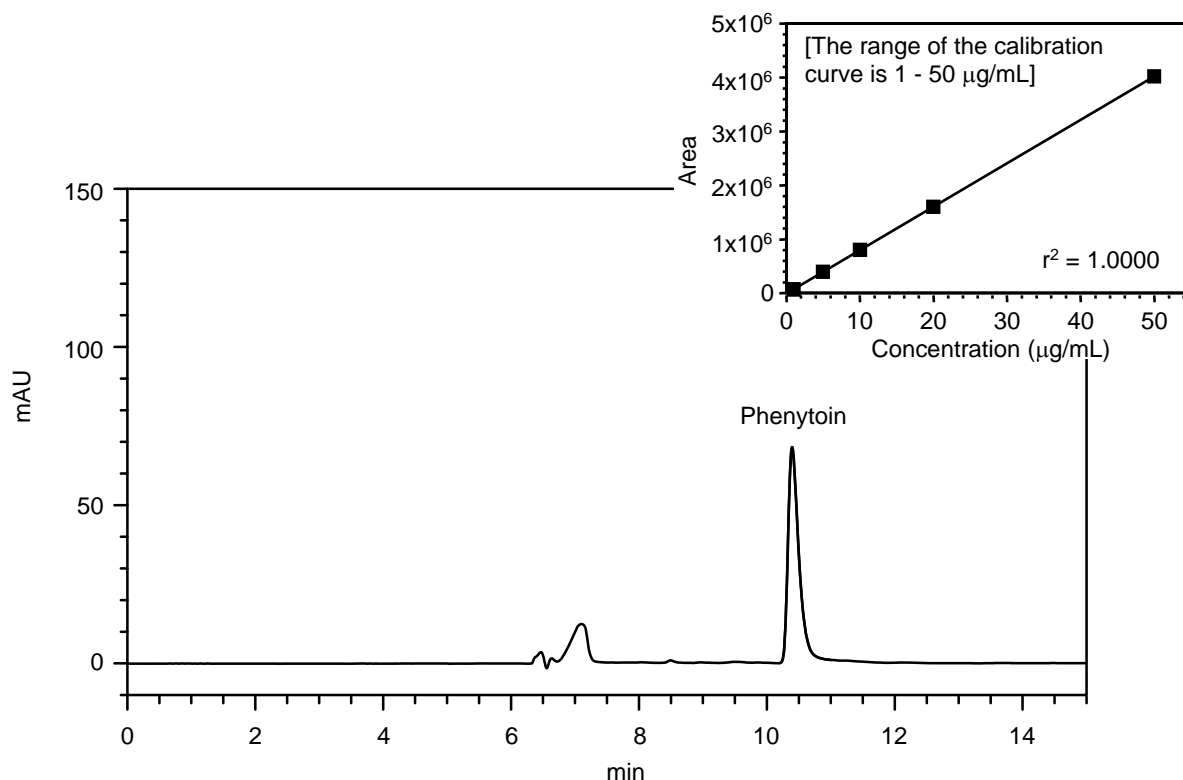


Analysis of Phenytoin using Column Switching System

When analyzing biological samples or samples with many contaminating substances, pretreatments such as deproteinization, contaminant removal, and concentration are necessary.

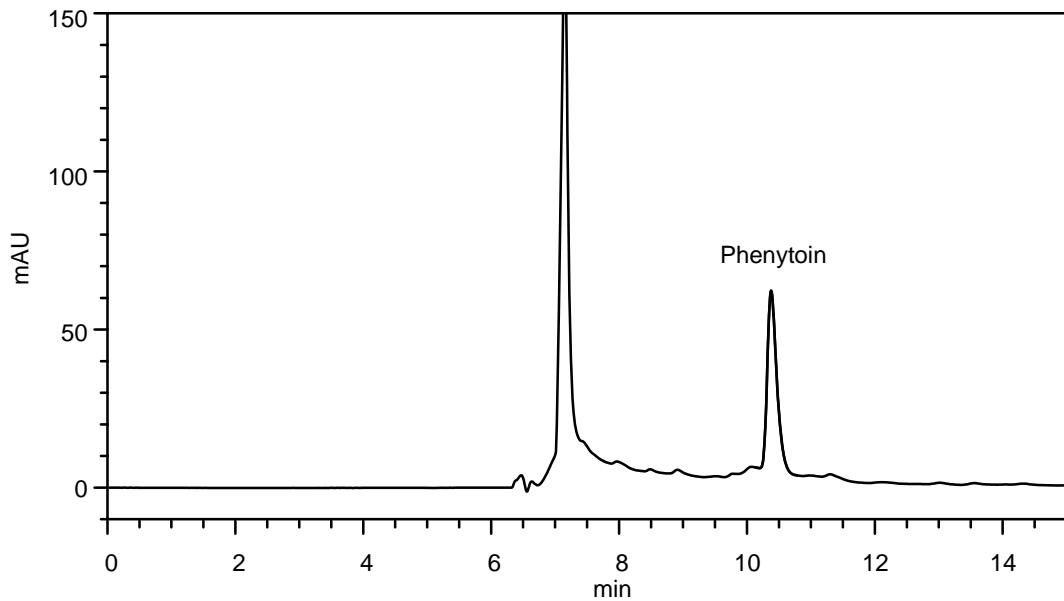
Chromaster system, when used with an optional valve, allows automated pretreatments by column switching method. Manual pretreatments can cause problems in the operational reproducibility and thus, the accuracy can be improved by automating the pretreatments.

An example of serum phenytoin analysis is introduced here. The column switching method with a pretreatment column for protein removal was used for deproteinization.



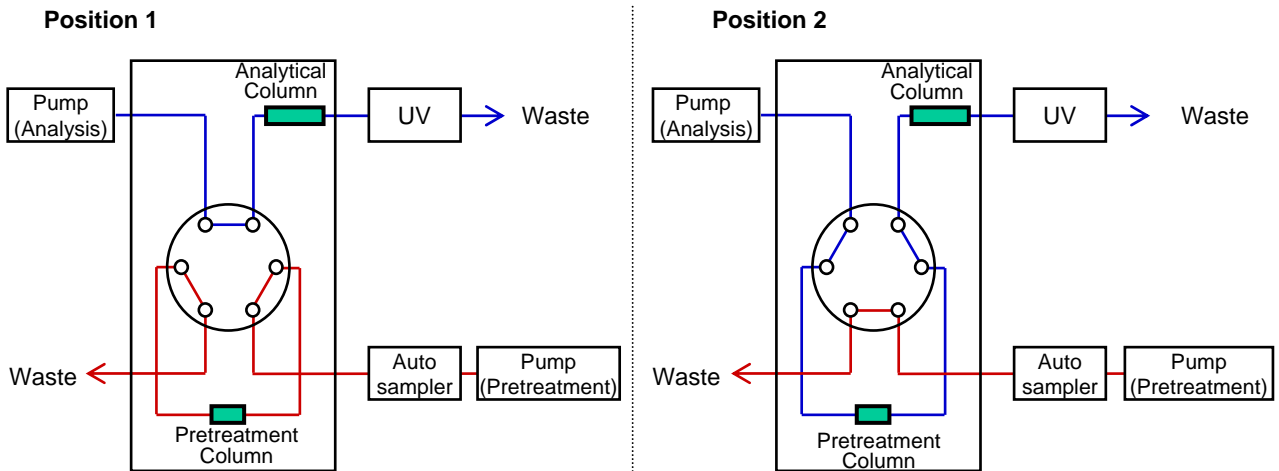
SAMPLE	20 µL of Std. Soln. (10 µg/mL)	PRESSURE	
PACKING MATERIAL	HITACHI LaChrom C18 (5 µm)	TEMPERATURE	40 °C
COLUMN SIZE	4.6 mm I.D. × 150 mm (P/N : 891-5050)	SEPARATION METHOD	Partition/Adsorption
ELUENT	(A) 50 mmol/L KH ₂ PO ₄ + K ₂ HPO ₄ (pH 6.9) / CH ₃ CN = 95 / 5 (v/v) (B) CH ₃ CN	DETECTOR	UV 210 nm
FLOW RATE	1.0 mL/min	INSTRUMENTS	Chromaster 5110 (Pump) × 2, Chromaster 5210 (Autosampler), Chromaster 5310 (Column Oven), Chromaster 5420 (UV-VIS Detector)
NOTE	Pretreatment column: MSpak PK-4A (4.0 mm I.D. × 10 mm)		
KEY WORDS	Bio/Medical Science/Food/Pharmaceutical, Medicine/Pharmaceutical, Medical Science/Pharmaceutical Science, Serum, Phenytoin, Antiepileptic Drug, Column Switching, UV-VIS Spectrometry, Health, Human Serum, Chromaster, LaChrom C18, Partition/Adsorption		High Performance Liquid Chromatograph (HPLC)
			Sheet No. LC100025-01

Analysis of Phenytoin in Human Serum using Column Switching System



[Sample Preparation Method]

- (1) Add phenytoin standard to human serum
- (2) Centrifuge and the supernatant is used as the sample for injection.



Time (min)	Pretreatment pump		Analysis pump		Valve position
	A (%)	B (%)	A (%)	B (%)	
0.0	95	5	65	35	1
5.0					2
8.0					1
10.0					1
10.1	35	65	35	65	1
20.0					1
20.1					1
30.0					1
30.1	95	5	65	35	1
40.0					1

High Performance Liquid
Chromatograph (HPLC)

Sheet No. LC100025-02