COSMOSIL

Biomolecule Analysis

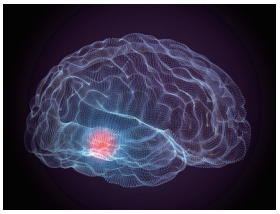
Technical Note

Various methods are used to identify and measure biomolecules in a clinical setting. In many cases, high performance liquid chromatography (HPLC) is a suitable method. However, some biomolecule analyses have difficulties, such as low retention or poor separation. The methods below have been developed for these difficult analytes.

Biologically active substances

(1) Catecholamines and other monoamines

Catecholamines are related to pheochromocytomas, neuroblastomas, and other illnesses. Screening methods include radioenzymatic assays and HPLC. COSMOSIL PBr can separate catecholamines and other monoamines using a simple method.



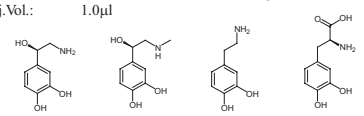
・**Catecholamines**

COSMOSIL Application Data

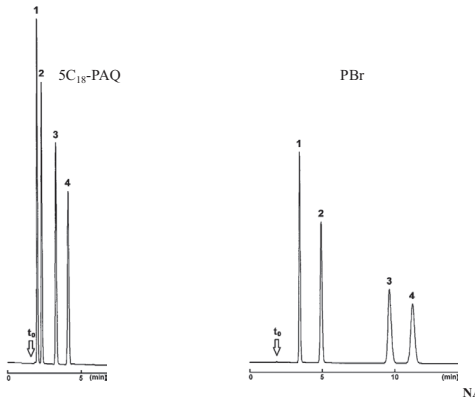
Column:
Column size: 4.6mmI.D.-150mm
Mobile phase: 20mmol/l Phosphate buffer(pH2.5)
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV280nm

Sample:		
	1; *L*-Noradrenaline	(0.5mg/ml)
	2; *L*-Adrenaline	(0.5mg/ml)
	3; Dopamine	(0.5mg/ml)
	4; *L*-DOPA	(0.5mg/ml)

Inj.Vol.: 1.0µl



1.*L*-Noradrenaline 2.*L*-Adrenaline 3.Dopamine 4.*L*-DOPA



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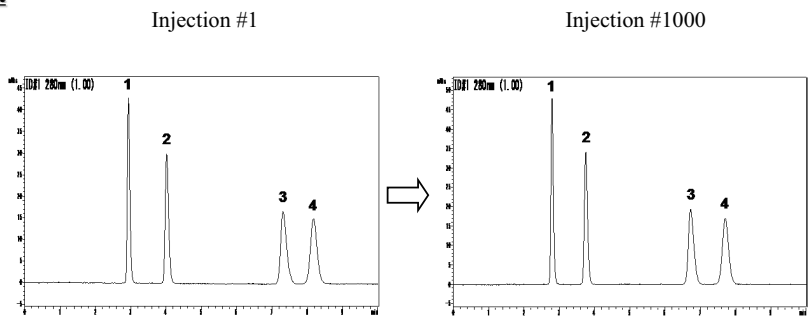
AP-1318

COSMOSIL Application Data

Column: PBr
Column size: 4.6mmI.D.-150mm
Mobile phase: 20mmol/l Phosphate buffer(pH2.5)
Flow rate: 1.0 ml/min
Temperature: 40°C
Detection: UV280nm

Sample:		
	1; *L*-Noradrenaline	(0.5mg/ml)
	2; *L*-Adrenaline	(0.5mg/ml)
	3; Dopamine	(0.5mg/ml)
	4; *L*-DOPA	(0.5mg/ml)

Inj.Vol.: 1.0µl



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AP-1322

・Analysis of monoamines in mouse brain tissue using LC-MS/MS

The standard chloroform/methanol extraction method for monoamines in mouse brain tissue resulted in peak distortion caused by impurities. For this experiment, a spin column was used for sample preparation. After preparation, the amounts of dopamine, noradrenaline and serotonin were quantified.

COSMOSIL Application Data

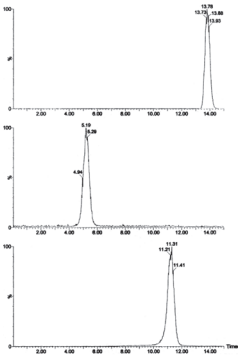
Column: PBr
Column size: 2.0mmI.D.-150mm
Mobile phase: 10mmol/l Ammonium Formate(pH 4.0)
Flow rate: 0.2 ml/min
Temperature: room temperature
Detection: ESI-MS/MS, Positive, MRM

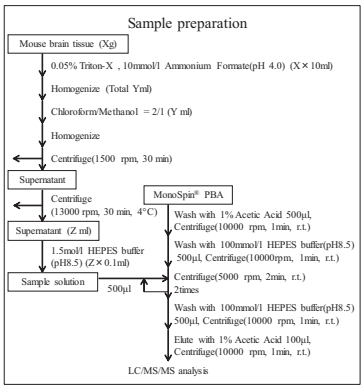
Sample: Monoamines in mouse brain
Dopamine
L-Noradrenaline
Serotonin

Serotonin
Q1 177/ Q3 115.1

L-Noradrenaline
Q1 169.9/ Q3 107

Dopamine
Q1 154.0/ Q3 91.0





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Data courtesy of Takuya Yamashita, Ph. D., Biochemical Toxicology Laboratory, School of Pharmaceutical Sciences, Kobe Gakuin University

Metabolite analysis

(1) Vitamin D

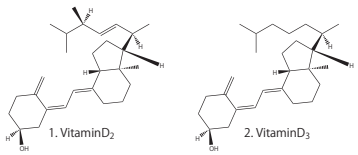
Vitamin D is involved in bone and other diseases. The simple Competitive Protein-Binding Assay (CPBA) is commonly used in clinical settings. However, it cannot measure different subtypes, and for this reason HPLC analysis is becoming more common in recent years. With HPLC, it is possible to simultaneously determine not only the 25-hydroxy vitamin D_2/D_3 metabolites, but also the C3 epimers and the vitamins themselves.

・Vitamin D_2/D_3 analysis by HPLC

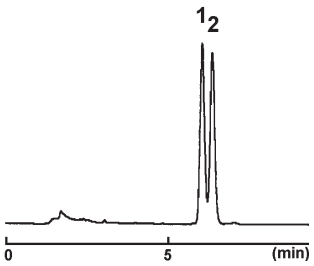
COSMOSIL Application Data

Column:
Column size: 4.6mmI.D.-150mm
Mobile phase: Methanol
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV265nm

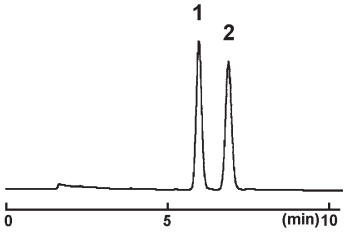
Sample: 1; Vitamin D_2 [Calciferol] (0.3µg)
2; Vitamin D_3 (0.1µg)



$5C_{18}$-MS-II



Cholester



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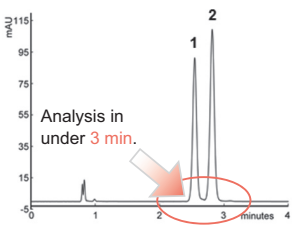
AP-1035

・Vitamin D_2/D_3 analysis by UHPLC

COSMOCORE Application Data

Column: COSMOCORE 2.6Cholester
Column size: 2.1mmI.D.-150mm
Mobile phase: Methanol
Flow rate: 0.4 ml/min
Temperature: 30°C
Detection: UV265nm

Sample: 1; Vitamin D_2 [Calciferol]
2: Vitamin D_3



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Data courtesy of a customer

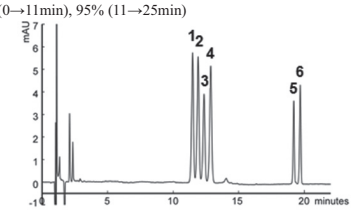
AP-1515

・Vitamin D metabolite analysis by UHPLC

COSMOCORE Application Data

Column: COSMOCORE 2.6Cholester
Column size: 2.1mmI.D.-150mm
Mobile phase: A; 0.1% Formic acid-H_2O
B; Methanol
B conc. 75→82% (0→11min), 95% (11→25min)
Flow rate: 0.3 ml/min
Temperature: 50°C
Detection: UV265nm

Sample:
1; *25*-Hydroxyvitamin D_3
2; *3-epi-25*-Hydroxyvitamin D_3
3; *25*-Hydroxyvitamin D_2
4; *3-epi-25*-Hydroxyvitamin D_2
5; Vitamin D_2 [Calciferol]
6: Vitamin D_3



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Data courtesy of a customer

AP-1514

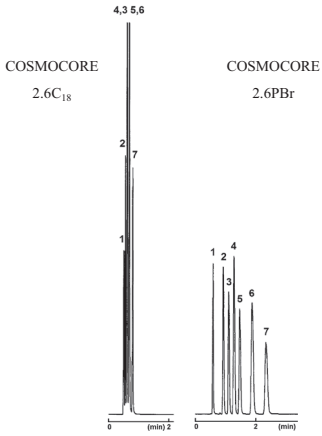
(2) Purine analysis (uric acid)

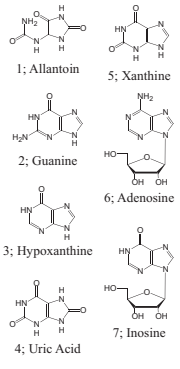
Uric acid is related to purine metabolism irregularities, renal dysfunction, and other illnesses. Commonly used screening methods include detection by reduction and uricase. With HPLC, uric acid metabolites (inosine, hypoxanthine, and xanthine) can be determined as well.

- **Nucleic acid derivatives**

COSMOCORE Application Data

Column: COSMOCORE **
Column size: 2.1mmI.D.-100mm
Mobile phase: Methanol/ 100mmol/l Phosphate buffer(pH2.5) = 10/90
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV240nm
Sample:
1; Allantoin (2.0mg/ml)
2; Guanine (0.05mg/ml)
3; Hypoxanthine (0.05mg/ml)
4; Uric Acid (0.1mg/ml)
5; Xanthine (0.2mg/ml)
6; Adenosine (0.2mg/ml)
7; Inosine (0.1mg/ml)
Inj. Vol: 1.0µl





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AP-1497

Drugs

(1) 5-Fluorouracil

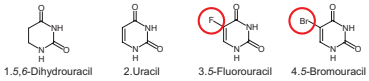
5-Fluorouracil is used to treat cancer by inhibition of thymidylate synthase. COSMOSIL PBr can retain uracil derivatives longer than C_{18} columns.

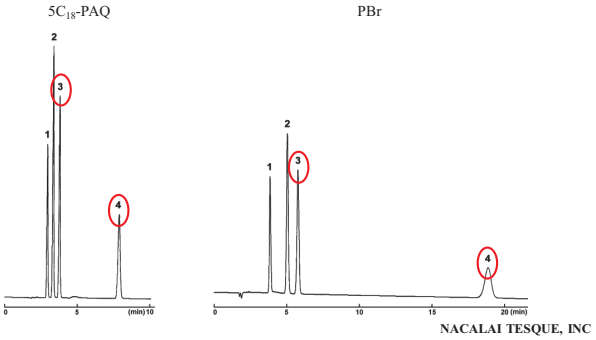
- **Uracil derivatives**

COSMOSIL Application Data

Column:
Column size: 4.6mmI.D.-150mm
Mobile phase: 20mmol/l Phosphate buffer(pH2.5)
Flow rate: 1.0 ml/min
Temperature: 30°C
Detection: UV210nm

Sample:
1; *5,6*-Dihydrouracil (0.005mg/ml)
2; Uracil (0.005mg/ml)
3; *5*-Fluorouracil (0.005mg/ml)
4; *5*-Bromouracil (0.005mg/ml)
Inj.Vol.: 10µl





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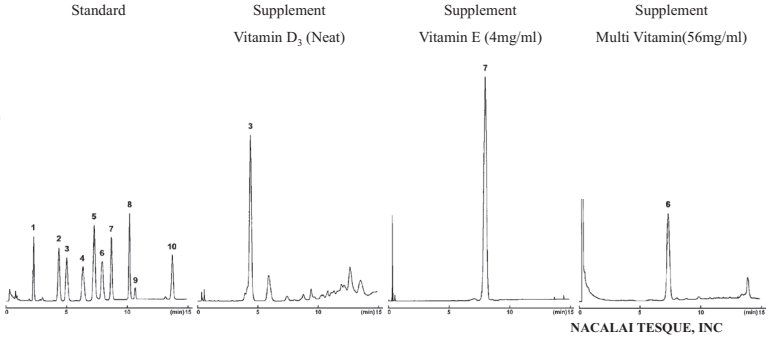
(2) Vitamin supplements

COSMOSIL 2.5Cholester completely separated the 10 fat-soluble vitamins below in 15 minutes.

- **Fat-soluble vitamins**

COSMOSIL Application Data

Column: 2.5Cholester
Column size: 2.0mmI.D.-50mm
Mobile phase: A; 0.1% TFA-Methanol/ H_2O = 90/10
B; 0.1% TFA-Methanol
B conc. 0%(0-5min)-100%(10min)-100%(15min)
Flow rate: 0.4 ml/min
Temperature: 40°C
Detection: UV280nm
Sample: (Standard)
1; Vitamin A Acetate, *all trans* (0.06mg/ml)
2; Vitamin D_2 [Calciferol] (0.30mg/ml)
3; Vitamin D_3 (0.06mg/ml)
4; Vitamin E Succinate [*D*-α-Tocopherol Succinate] (0.60mg/ml)
5; Vitamin K_2 [Menatetrenone] (0.18mg/ml)
6; Vitamin E [*DL*-α-Tocopherol] (0.60mg/ml)
7; Vitamin E Acetate [*DL*-α-Tocopherol Acetate] (0.60mg/ml)
8; *trans*-Vitamin K_1 (0.18mg/ml)
9; *cis*-Vitamin K_1
10; Vitamin A Palmitate (0.18mg/ml)
Inj. Vol: 2.5µl



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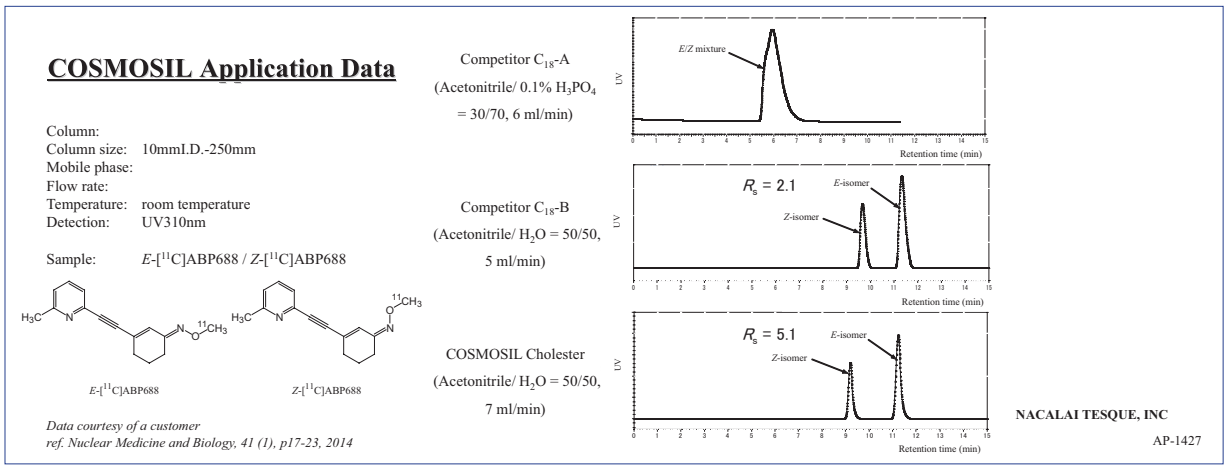
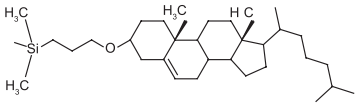
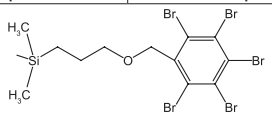
AP-1262

(3) Radiopharmaceuticals (PET)

[*11*C]ABP688 is a metabotropic glutamate receptor subtype 5-selective positron emission tomography (PET) ligand. Presence of the *Z* isomer may reduce detection sensitivity. Using COSMOSIL Cholester yields higher resolution compared to C_{18} columns, resulting in higher efficiency for preparative separations.



Specifications

Packing Material	COSMOSIL Cholester	COSMOSIL 2.5Cholester	COSMOCORE 2.6Cholester	COSMOSIL PBr	COSMOCORE 2.6PBr
USP Code	L101			-	
Silica Gel	High Purity Porous Spherical Silica		Core-shell type silica	High Purity Porous Spherical Silica	Core-shell type silica
Particle Size	5 µm	2.5 µm	2.6 µm	5 µm	2.6 µm
Stationary Phase					
	Cholesteryl Group			Pentabromobenzyl group	
pH Range	2 ～ 7.5				
Maximum Puressure	20 MPa (15 MPa for 10 mm I.D. or greater)	30MPa	60MPa	20 MPa (15 MPa for 10 mm I.D. or greater)	60MPa
Connection Type	HPLC Type		UHPLC Type	HPLC Type	UHPLC Type

Ordering Information

COSMOSIL Cholester

Column Size I.D. x Length (mm)	Product Number
2.0 × 150	05971-11
4.6 × 150	05976-61
10 × 250	05979-31

COSMOSIL 2.5Cholester

Column Size I.D. x Length (mm)	Product Number
2.0 × 50	09000-01
2.0 × 75	09047-11
2.0 × 100	09048-01

COSMOCORE 2.6Cholester

Column Size I.D. x Length (mm)	Product Number
2.1 × 50	12859-81
2.1 × 100	12861-31
2.1 × 150	12862-21

COSMOSIL PBr

Column Size I.D. x Length (mm)	Product Number
2.0 × 150	12392-81
4.6 × 150	12394-61
10 × 250	12397-31

COSMOCORE 2.6PBr

Column Size I.D. x Length (mm)	Product Number
2.1 × 50	13693-11
2.1 × 100	13695-91
2.1 × 150	13697-71

For research use only, not intended for diagnostic or drug use.

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The quality for certainty.

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