International Symposium on 22nd MDO and the 33rd Annual Meeting of JSSX
JSSX Award Winner's Presentation
2018.10.2 Kanazawa Art Hall

Pharmacokinetic Studies based on Clinical Chemistry

臨床化学を基盤とした薬物動態研究

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東北大学病院薬剤部

What is “Clinical Chemistry”?

Clinical chemistry is an academic discipline that sees practical application in the clinical setting. In addition to forming the basis of services providing chemical analysis results for medical care in the daily clinical setting, it aids the elucidation of disease etiologies and pathologies and contributes to treatment and prevention. Thus, having various academic and practical aspects and including both analytical chemistry and clinical medicine, clinical chemistry is very wide ranging, and there is much interaction with other academic disciplines. Therefore, it does not only involve researchers in university and other research institutions but also those in various research laboratories such as those in hospitals and individual clinical departments, and industry research institutions.

(Japan Society of Clinical Chemistry)

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Why I started the study using fluorescent probes

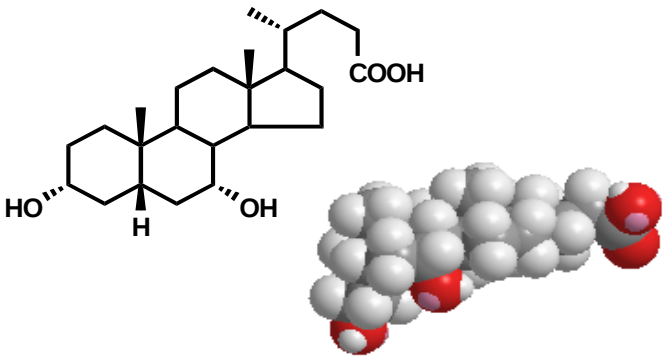
- Bioimaging research has been actively conducted to see various biological reactions occurring in body.
- Most of them aim to dynamically observe movement of specific genes or proteins.
- On the other hand, bioimaging research on membrane permeation mechanism of endogenous small molecules and drugs has not been developed.

I thought that…

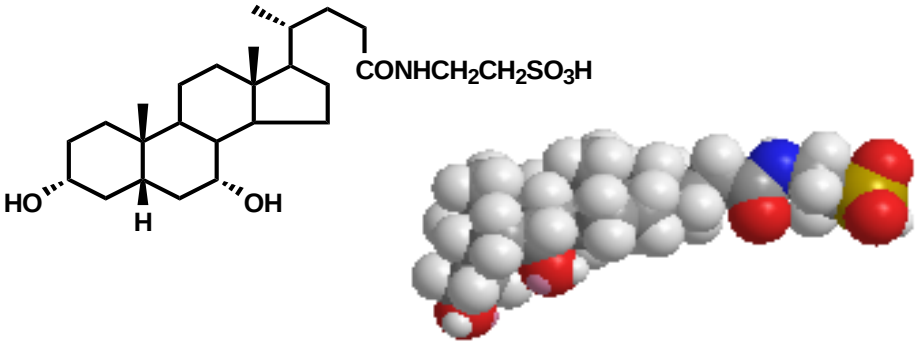
Imaging of membrane transport of low molecular compounds can be expected to observe transporter function over time and to be applicable to high throughput screening.

Chemical structure of fluorescent bile acid

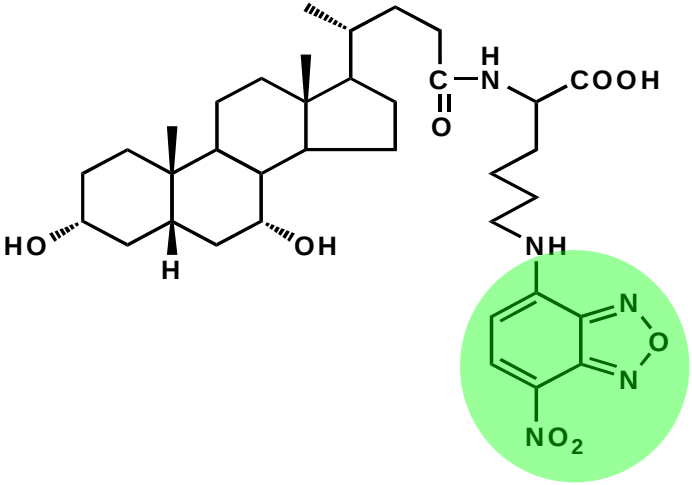
Chenodeoxycholic acid

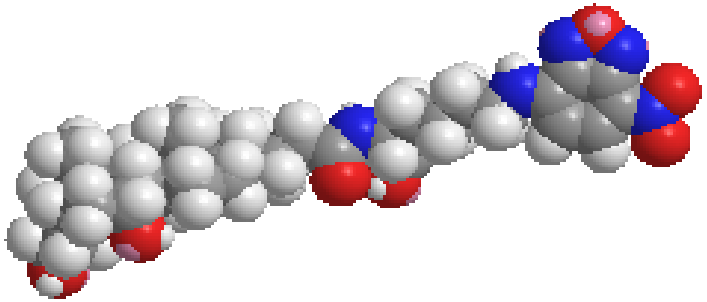


Taurochenodeoxycholate



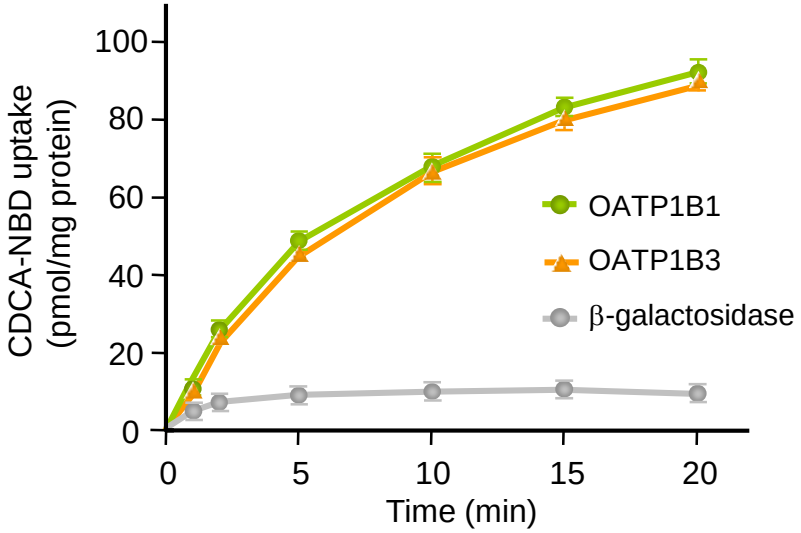
CDCA-(*N*ε-NBD)-lysine (CDCA-NBD)



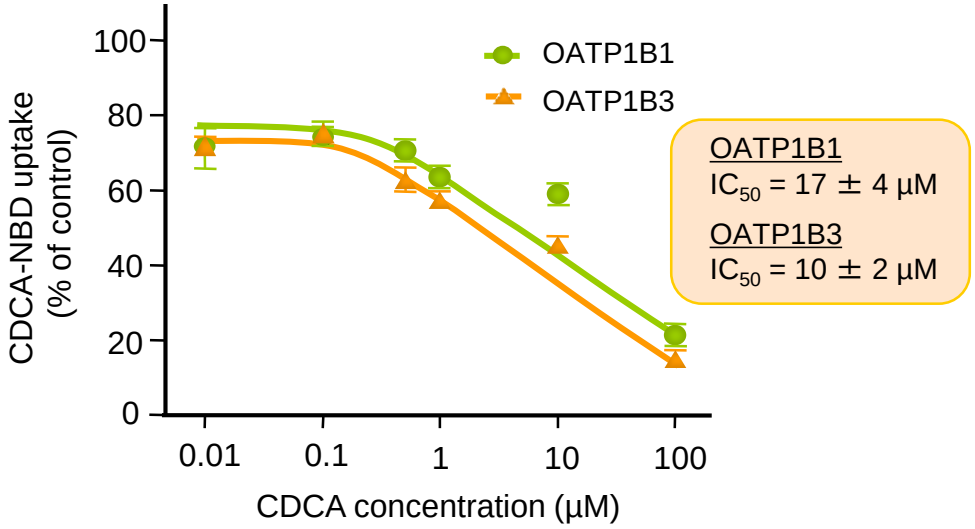


Transport of CDCA-NBD by OATP1B1- and OATP1B3-overexpressing cells

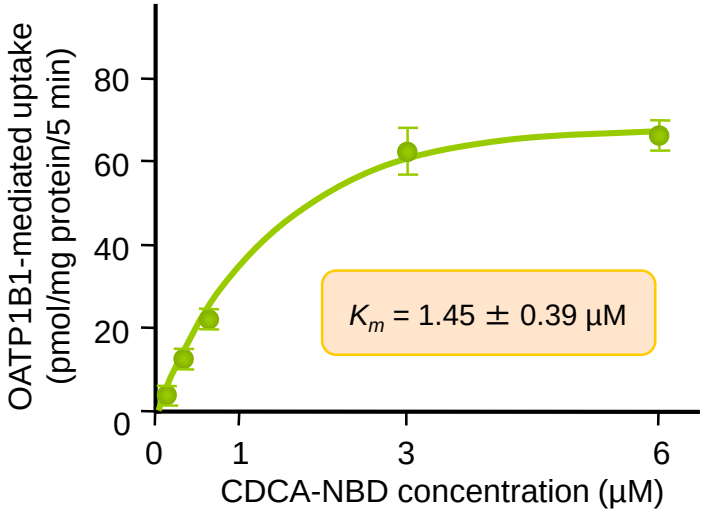
Time-dependent uptake

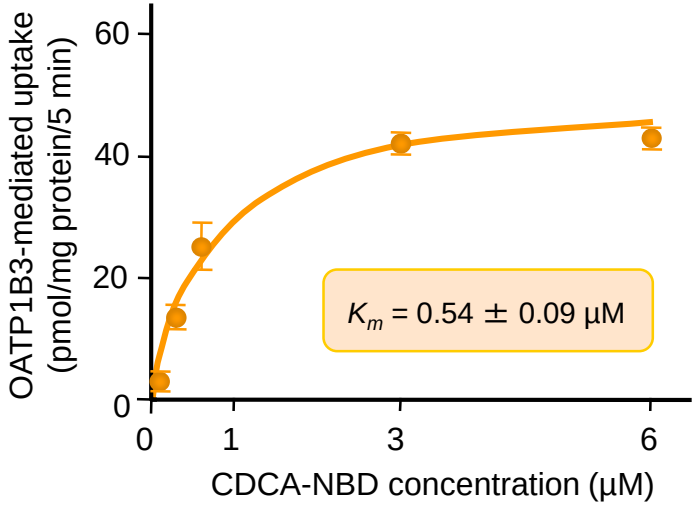


Concentration-dependent CDCA inhibition



Concentration-dependent uptake

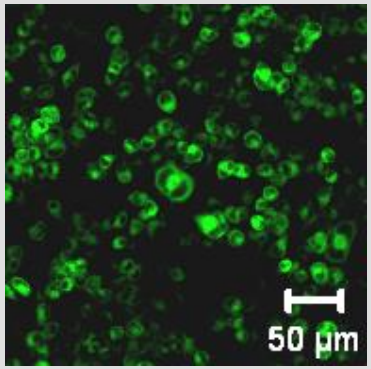


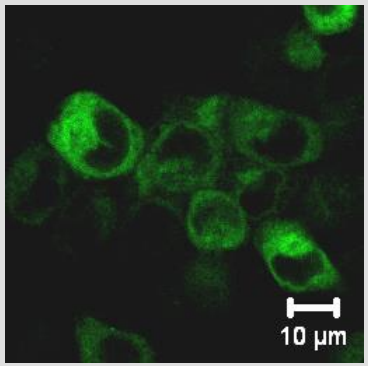


Yamaguchi H et al. *J Lipid Res* (2006)

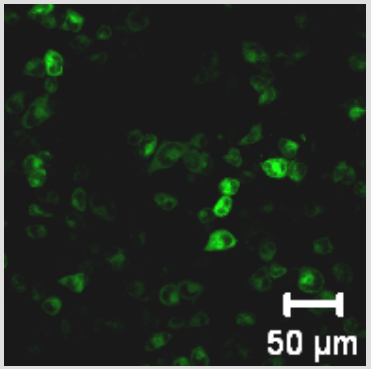
Visualization of CDCA-NBD transport via OATP1B1 and OATP1B3

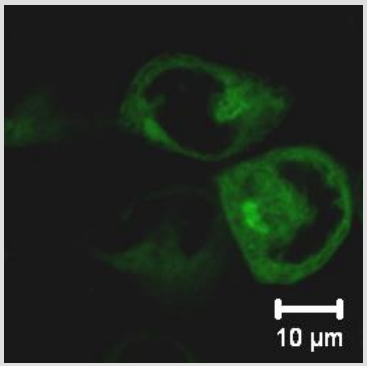
HepG2/OATP1B1



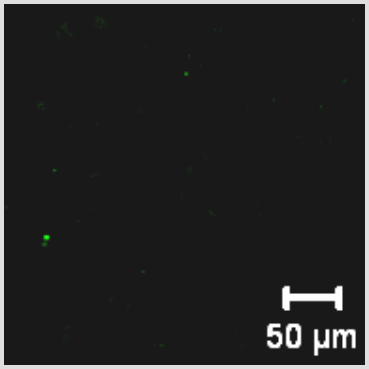


HepG2/OATP1B3





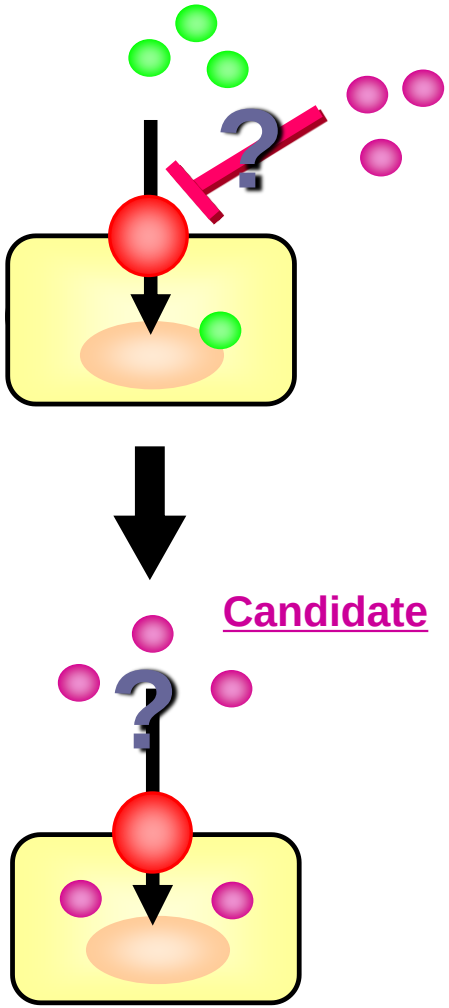
HepG2/β-galactosidase



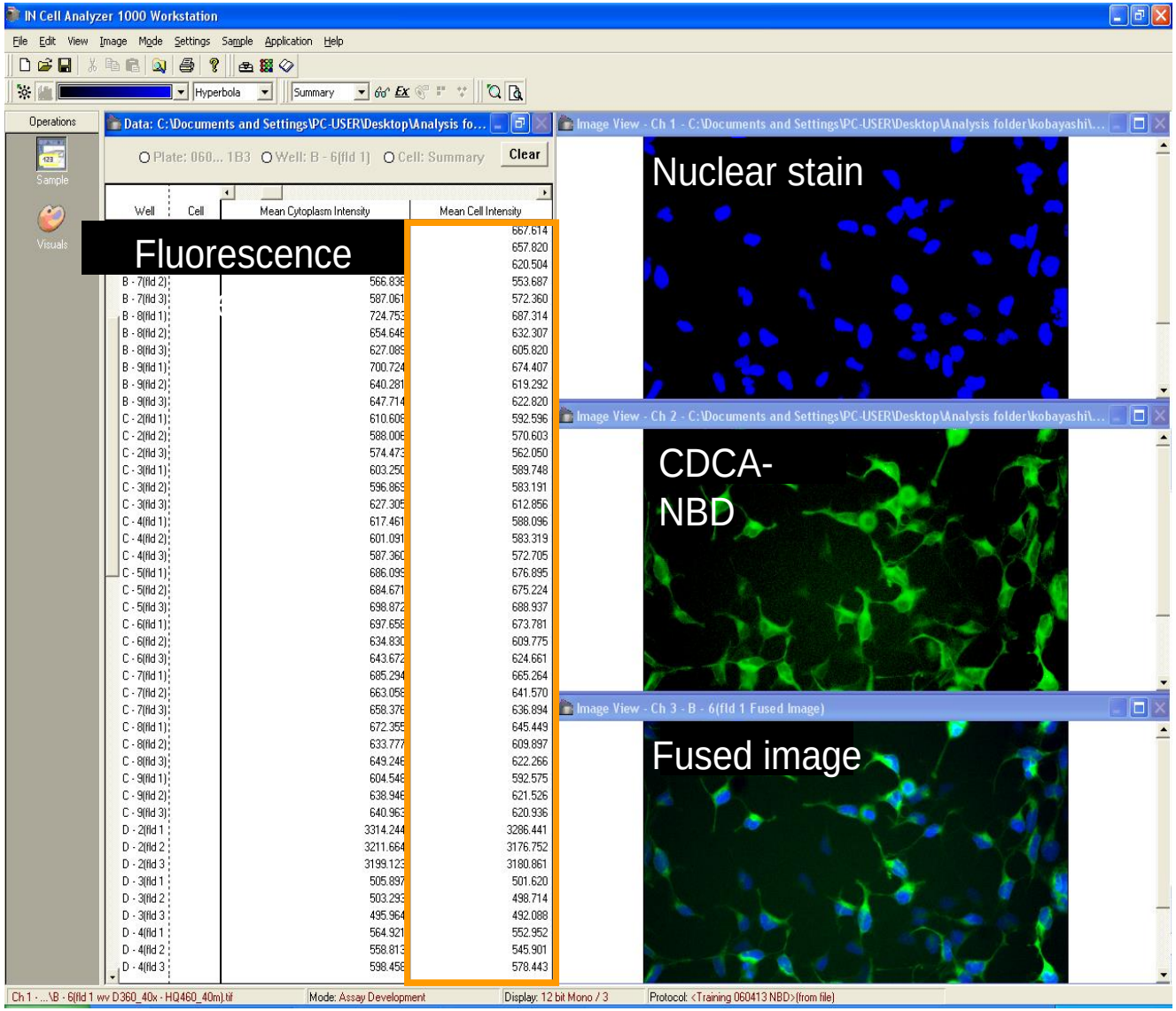
Yamaguchi H et al. *J Lipid Res* (2006)

Drug screening system using fluorescent probe

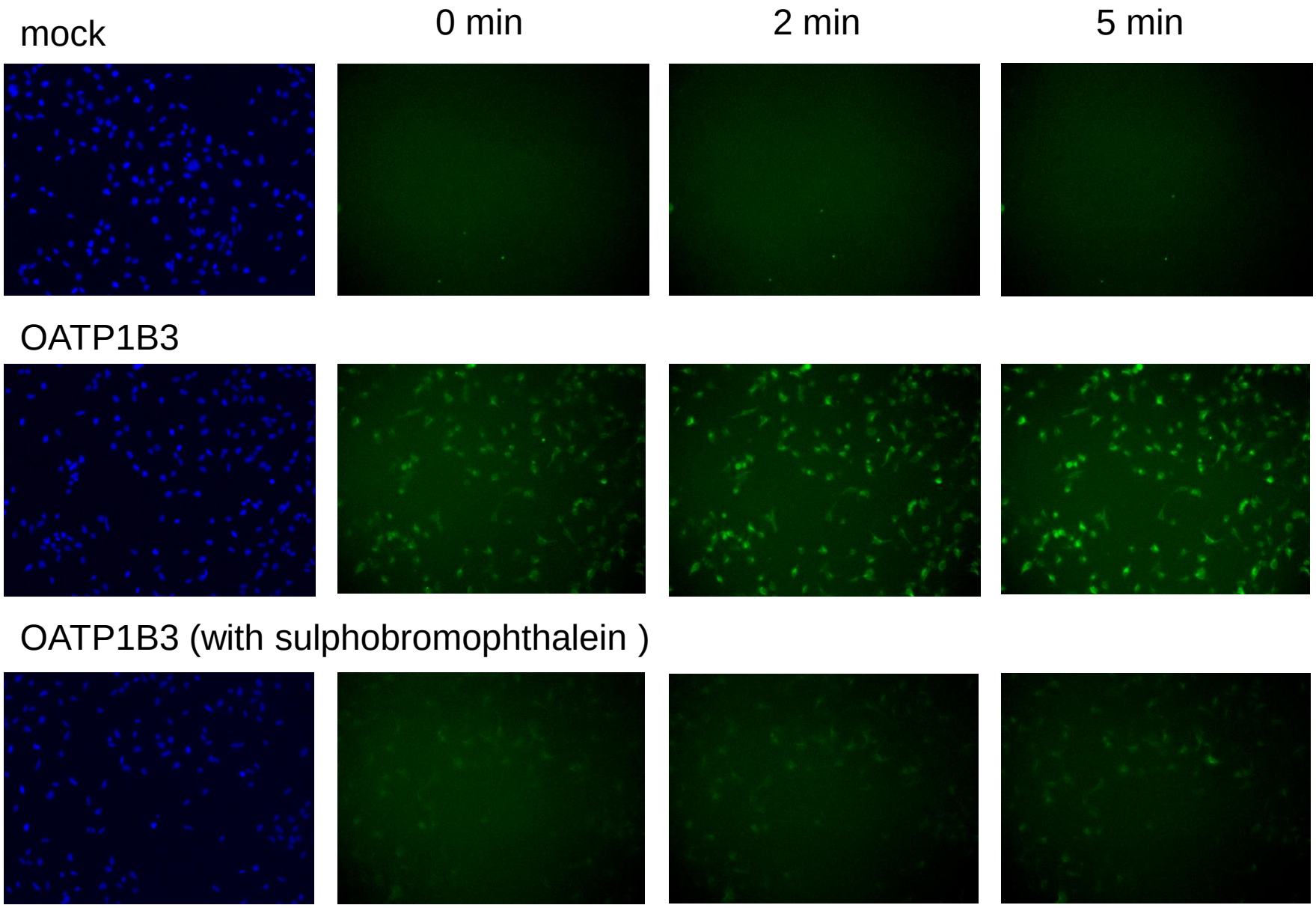
Fluorescent probe



IN Cell Analyzer 1000 (GE Healthcare)

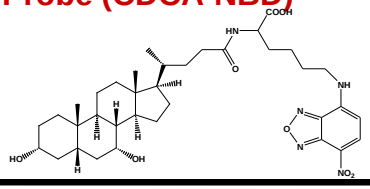


Images of OATP1B3-mediated CDCA-NBD transport

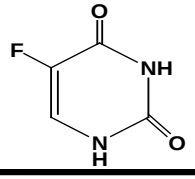


Tested drugs for the screening of candidate of OATP1B3 substrate

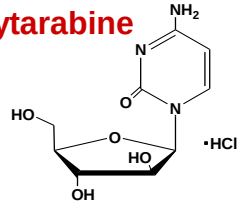
Probe (CDCA-NBD)



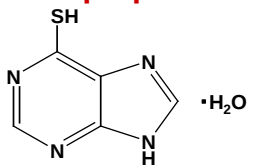
1 5-Fluorouracil



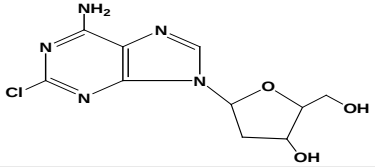
2 Cytarabine



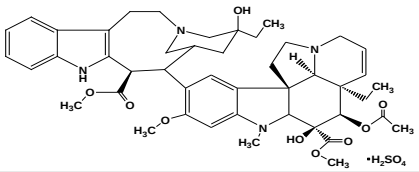
3 6-Mercaptopurine



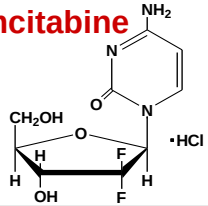
4 Cladribine



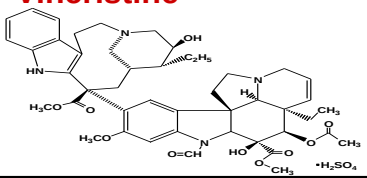
5 Vinblastine



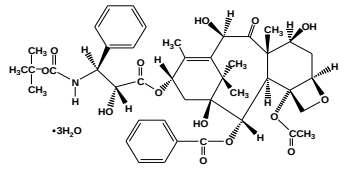
6 Gemcitabine



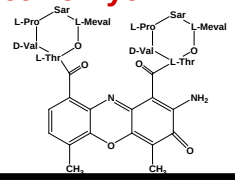
7 Vincristine



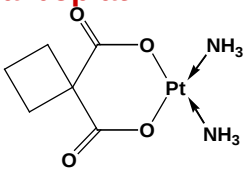
8 Docetaxel



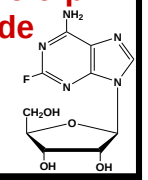
9 Actinomycin D



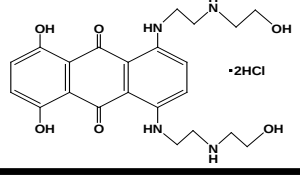
10 Carboplatin



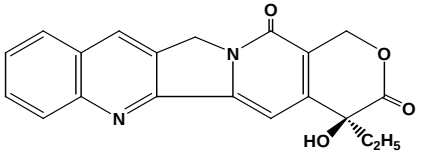
11 2-Fluoroadenine-9-β-D-arabinofuranoside



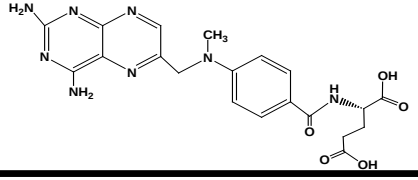
12 Mitoxantrone



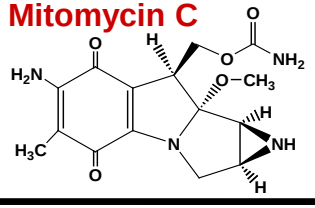
13 Camptothecin



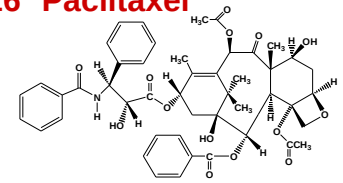
14 Methotrexate



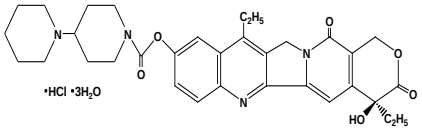
15 Mitomycin C



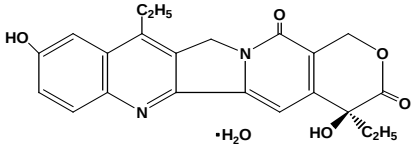
16 Paclitaxel



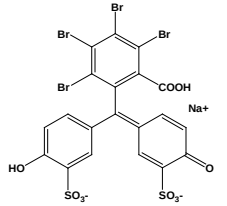
17 Irinotecan



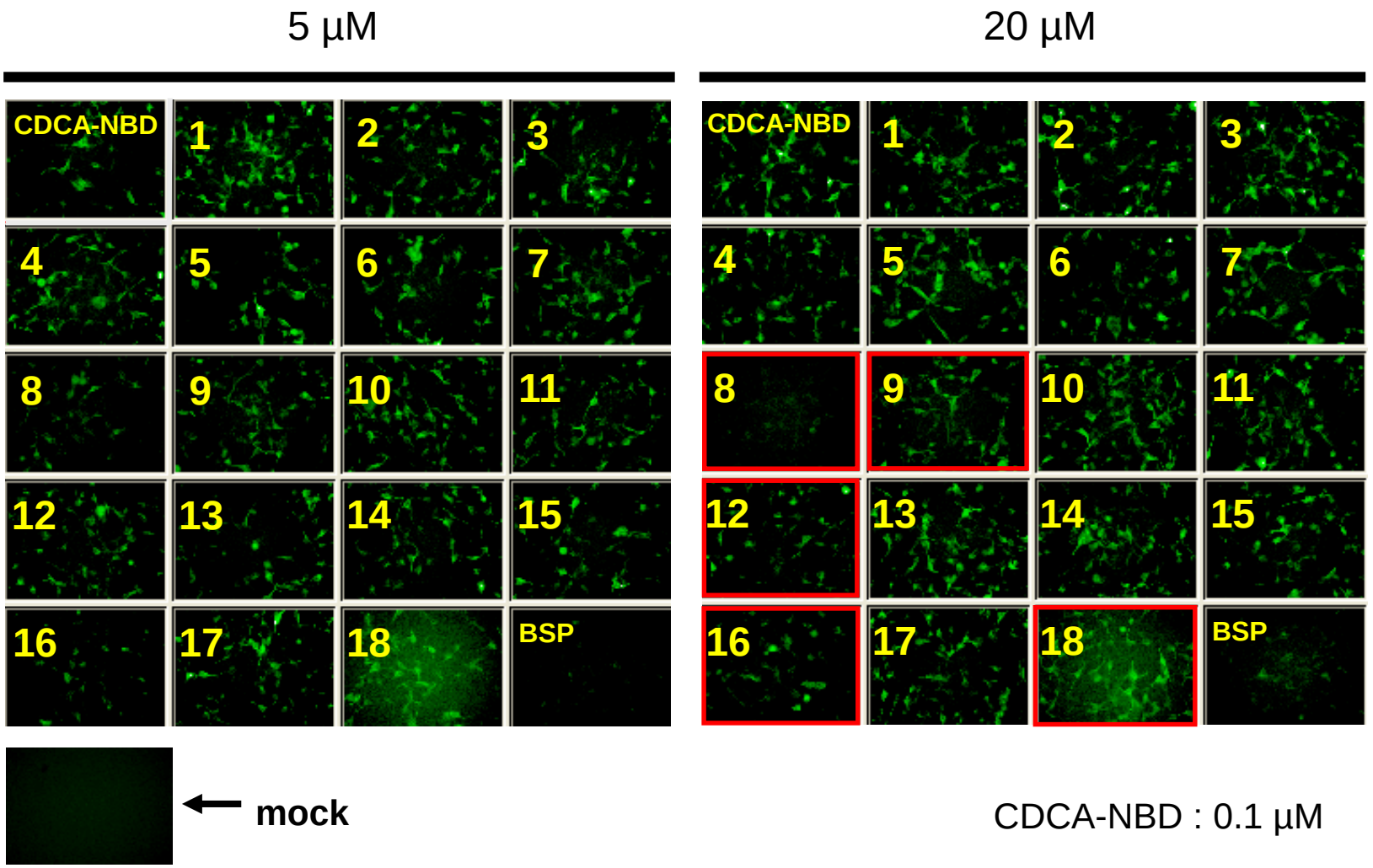
18 SN-38



BSP

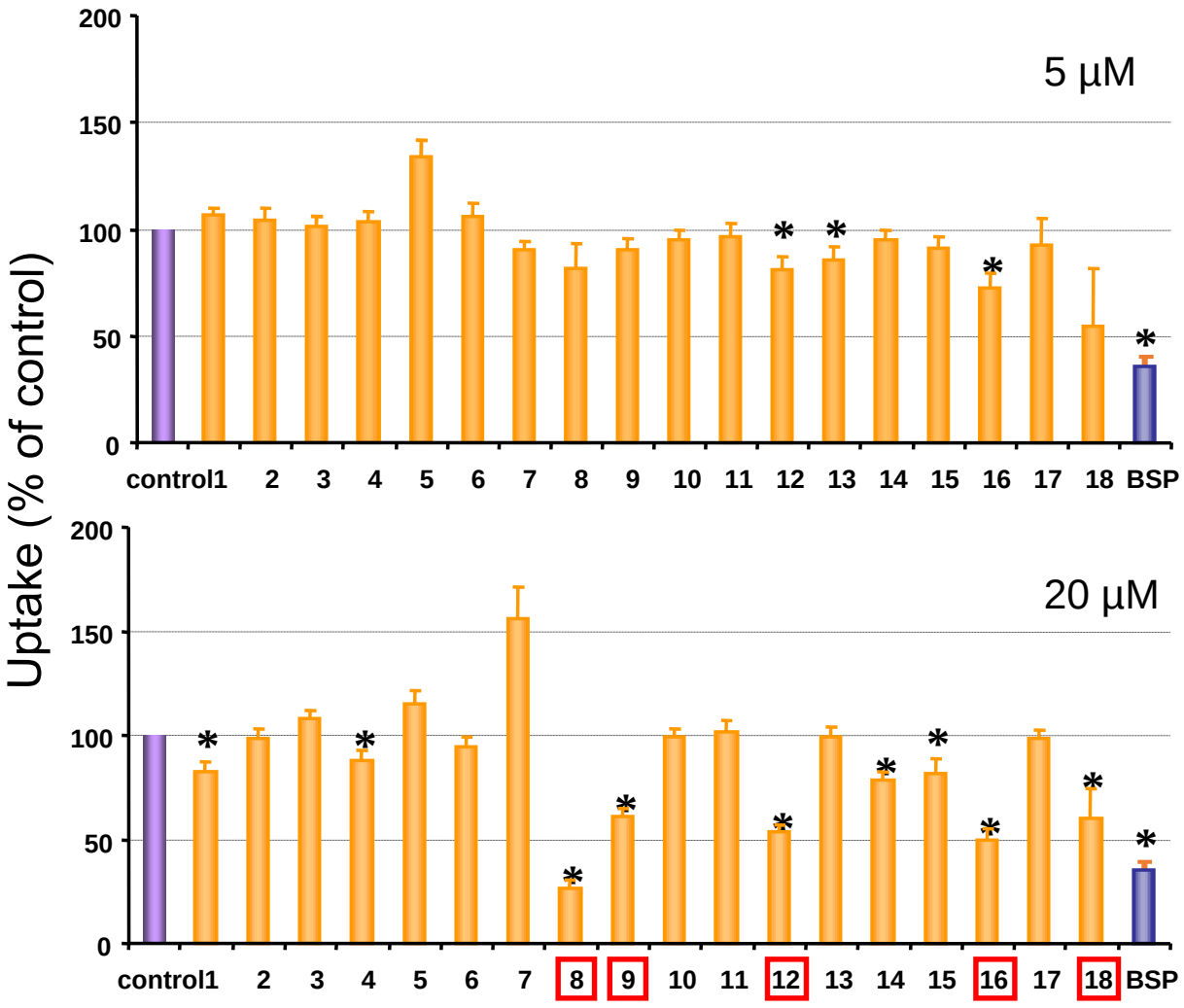


Effect of anticancer drugs on OATP1B3-mediated CDCA-NBD transport



Yamaguchi H et al. *Cancer Lett*. (2008)

Effect of anticancer drugs on OATP1B3-mediated CDCA-NBD transport

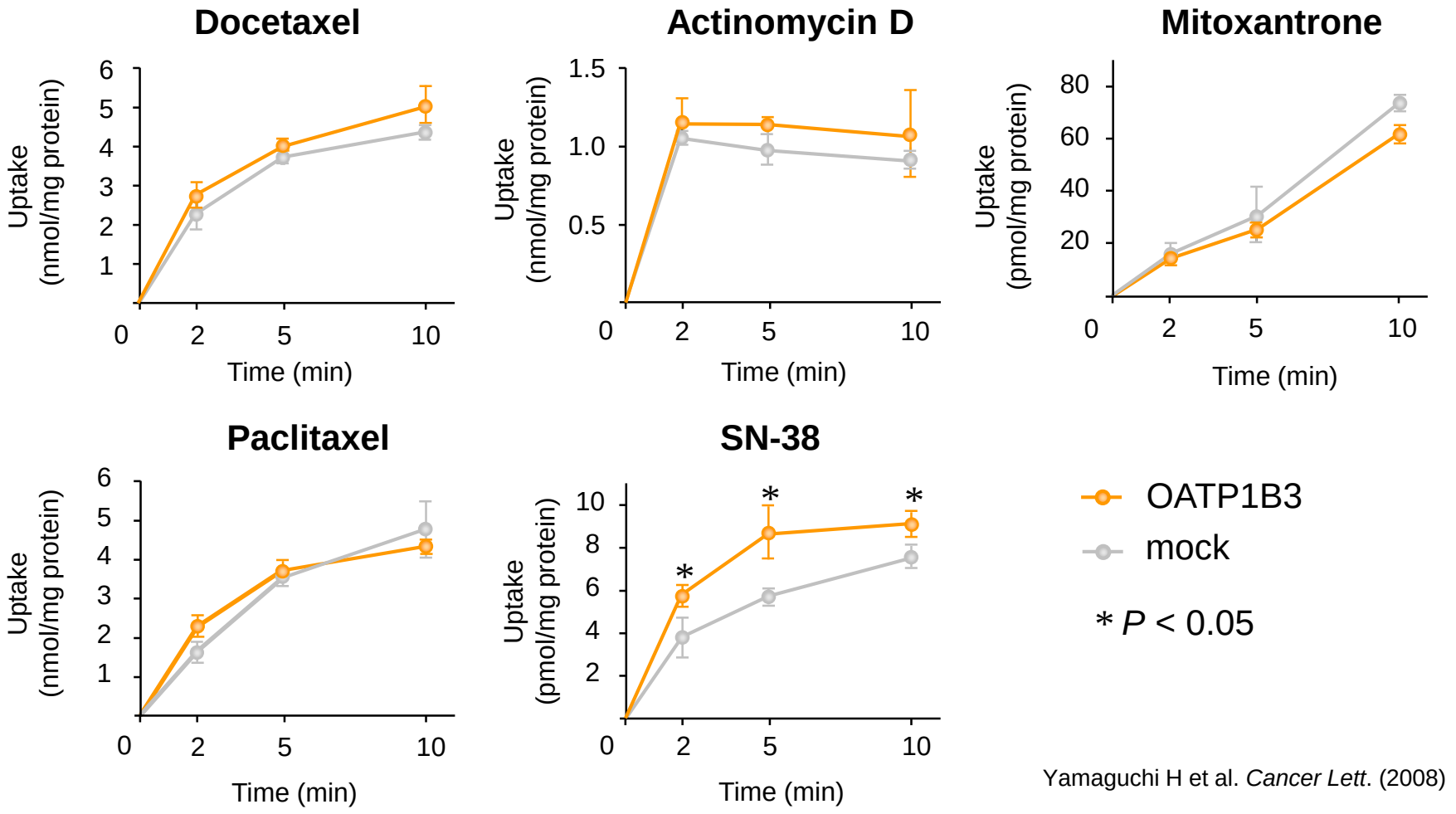


1	5-Fluorouracil
4	Cladribine
8	Docetaxel
9	Actinomycin D
12	Mitoxantrone
13	Camptothecin
14	Methotrexate
15	Mitomycin C
16	Paclitaxel
18	SN-38

* $P < 0.05$

Control = OATP1B3 - mock (CDCA-NBD intensity) = 100%

Transport of substrates candidate by OATP1B3

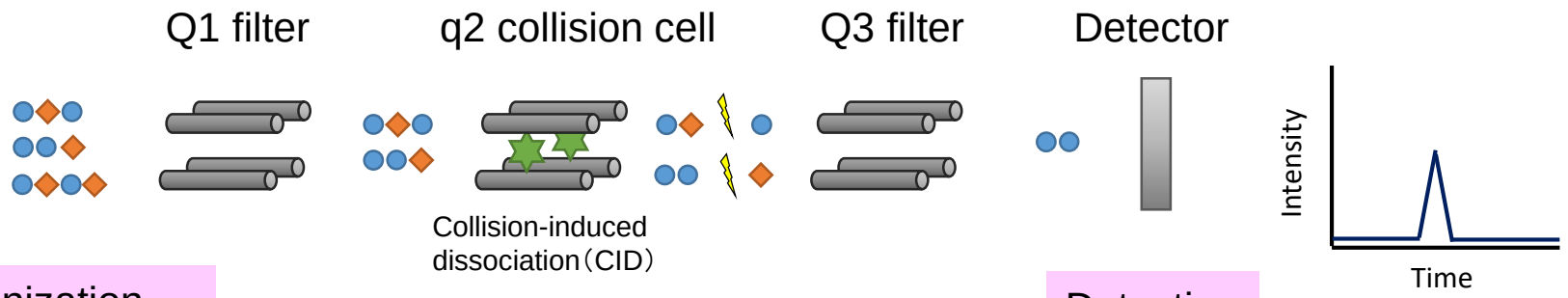


Yamaguchi H et al. *Cancer Lett*. (2008)

Fluorescent probes are useful for not only the visualization of transporter function but also the detecting the transporter-mediated drug-drug interaction and seeking the candidates of transporters.

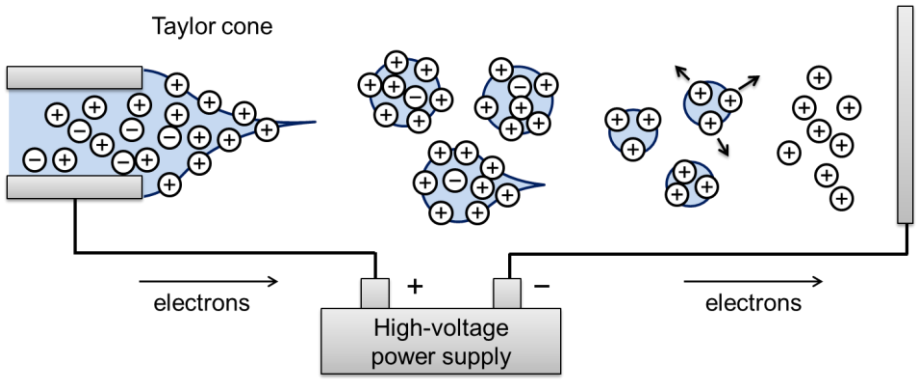
Contents

Quantification of low molecular weight compounds by LC/ESI-MS/MS



In mass spectrometry, the linear ranges of responses are relatively narrow due to saturation of ionization and/or detection by an electron multiplier.

Principle of electrospray ionization (ESI)



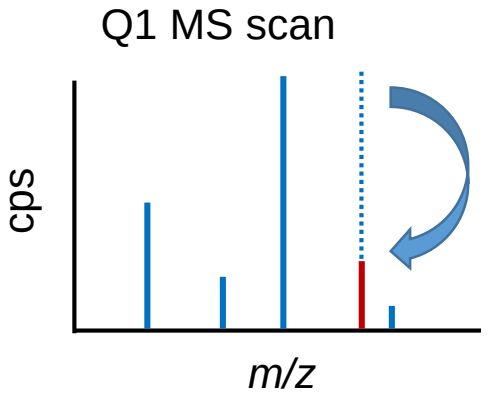
Ionization saturation is particularly common in ESI.

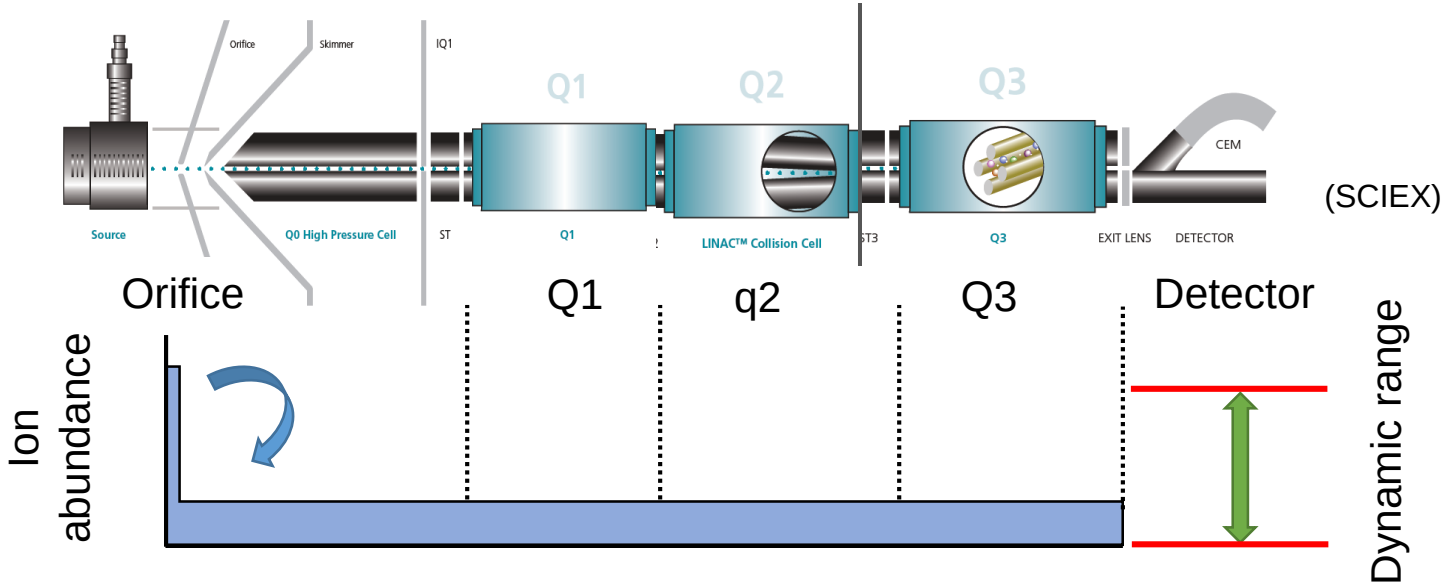
When linearity cannot cover the quantitative range due to saturation, we must reduce the injection volume or dilute the samples.

Therefore, multiple analyses are required when analyzing more than two analytes with extremely different responses, such as a drug and its metabolites.

In-source collision-induced dissociation (CID)

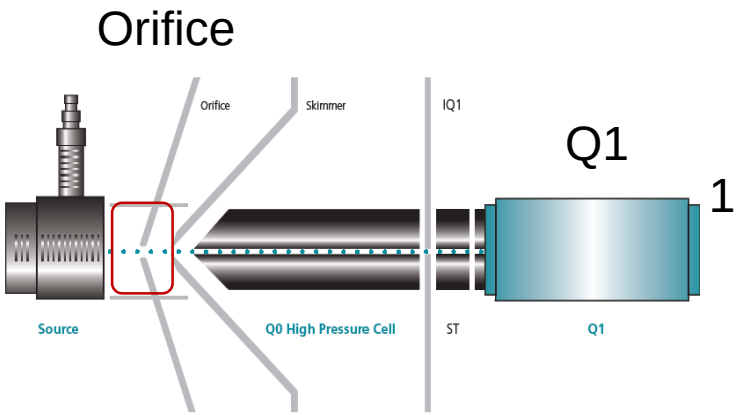
- Monitor precursor ions reduced by CID
- Limit the amount of ions after orifice
- Ion amount can be arbitrarily controlled by applied voltage

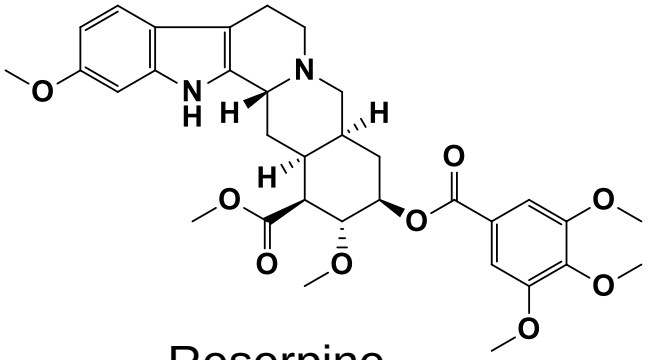




In-source CID has been used for structural analysis in qualitative analysis and noise reduction by decomposition of contaminants in quantitative analysis.

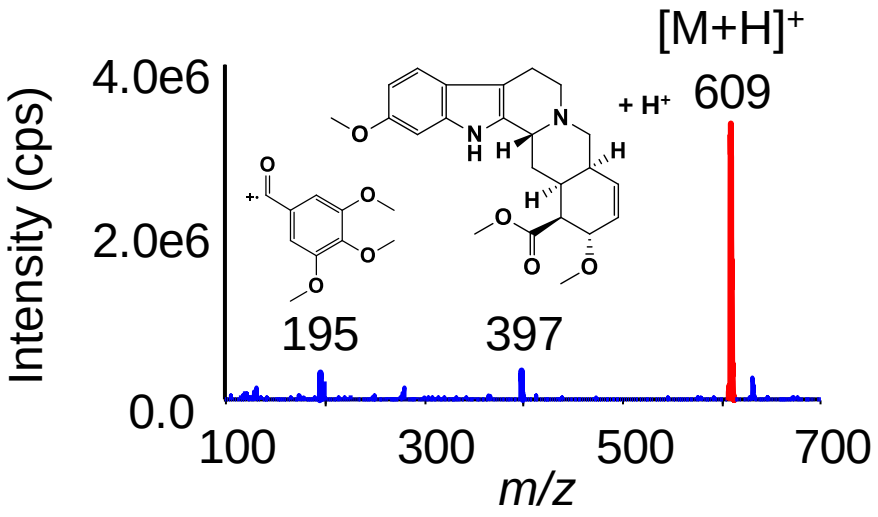
Principle of in-source CID



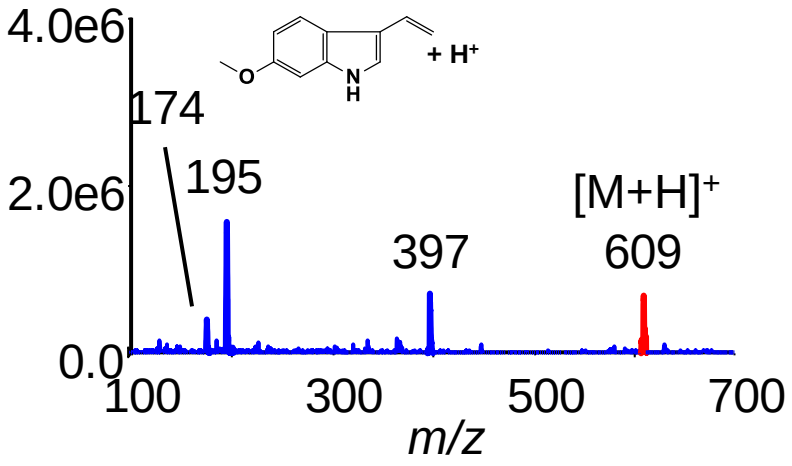


Reserpine

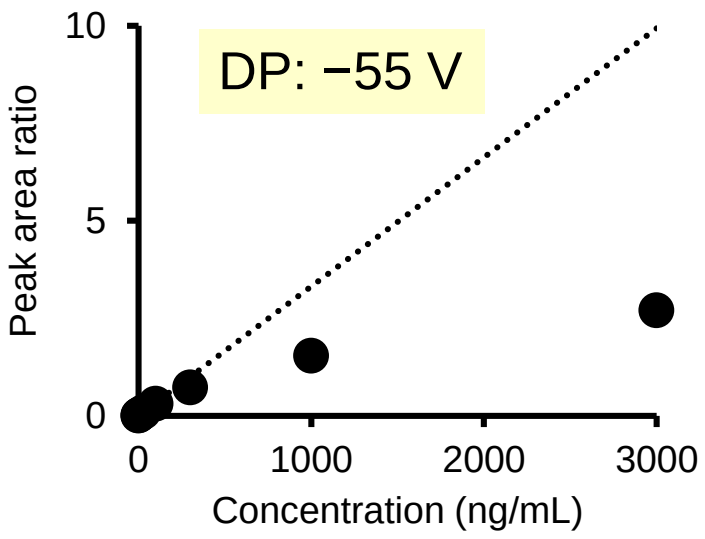
Declustering potential（DP）: 135 V

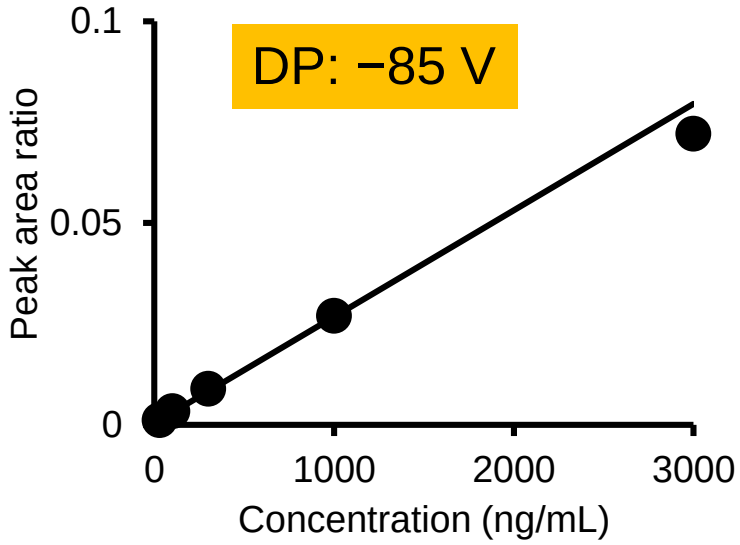


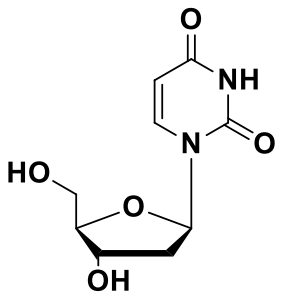
DP: 175 V



Shifting the linear range of dUrd by in-source CID







2'-Deoxyuridine (dUrd)
$C_9H_{12}N_2O_5$
Exact mass
228.07
Log P -1.5
p*K*a 9.7

$y = 3.3 \times 10^{-3} x + 6.0 \times 10^{-4}$ ($r = 1.000$)

$y = 2.0 \times 10^{-5} x + 1.4 \times 10^{-3}$ ($r = 0.999$)

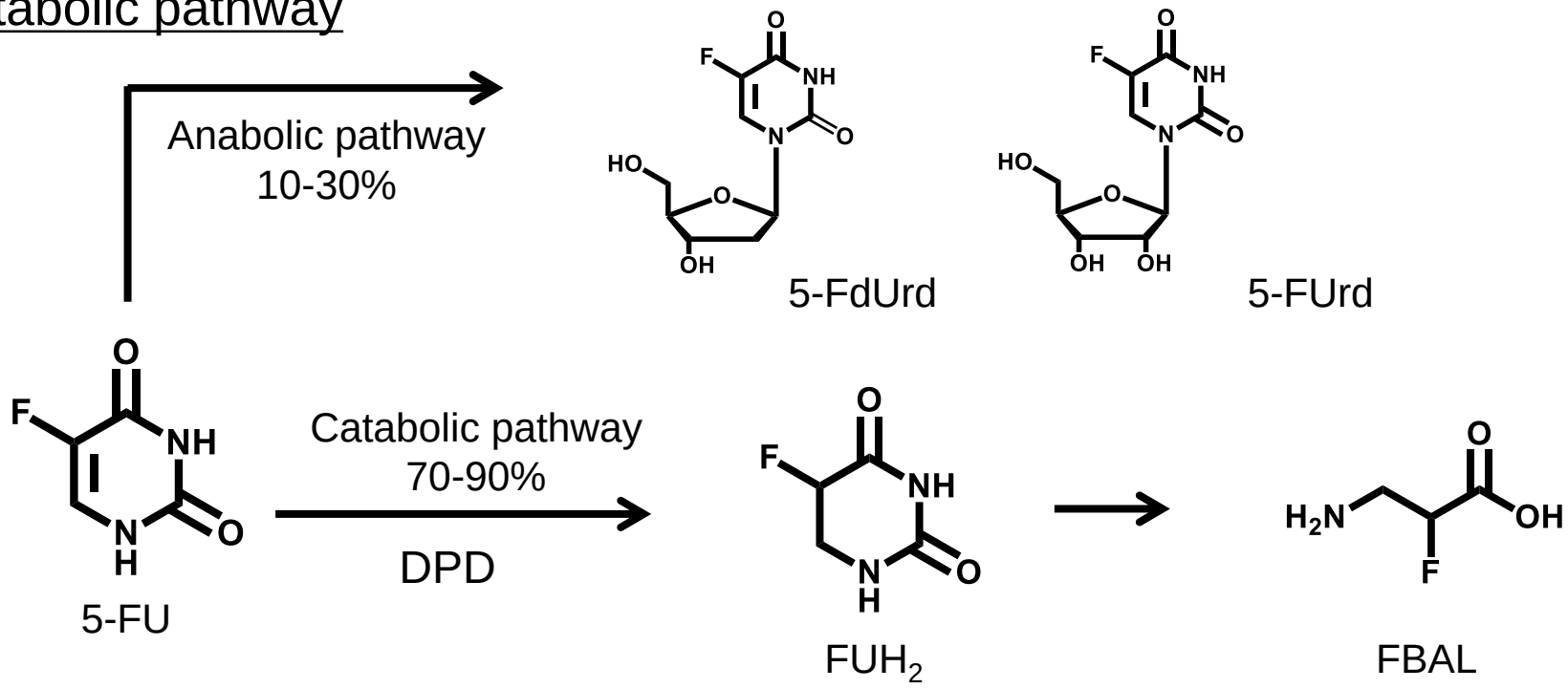
Linear range : **1-100 ng/mL** → Linear range : **30-3,000 ng/mL**

Linear range shifted by 30 times

Ishii H et al. *Chem Pharm Bull*. (2016)

Application to determination method for 5-FU and its metabolites

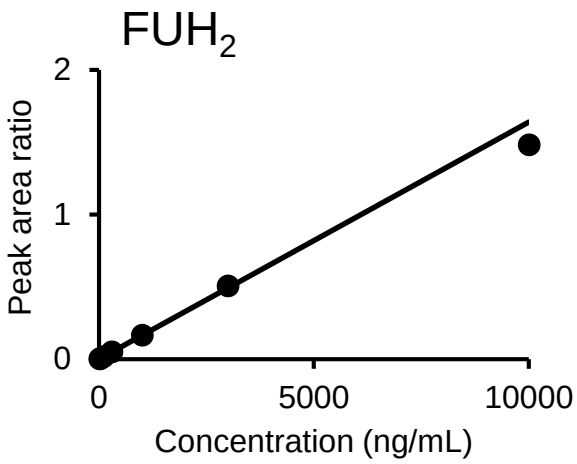
Metabolic pathway

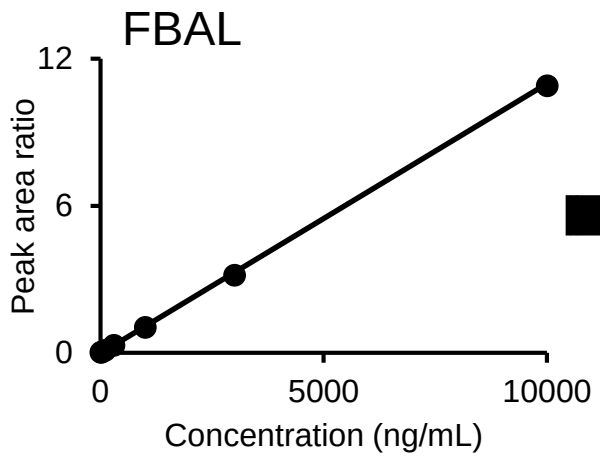


Quantitative range of 10-10,000 ng/mL is required for pharmacokinetic analysis. However, simultaneous measurement is difficult because physical properties and detection intensity are largely different.

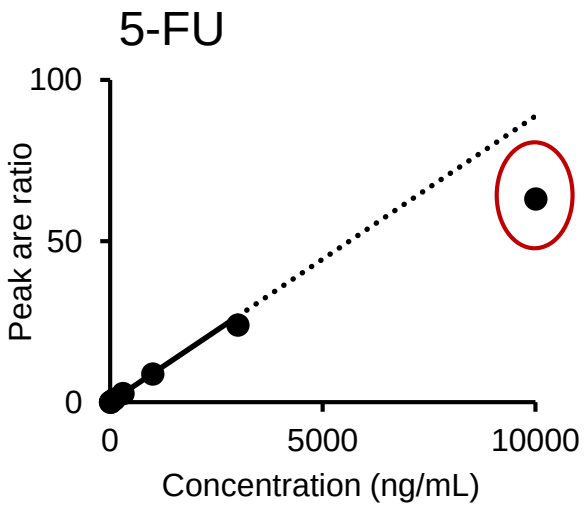
5-Fluorouracil (5-FU), 5-Fluorodeoxyuridine (5-FdUrd), 5-Fluorouridine (5-FUrd), 5,6-Dihydrofluorouracil (FUH_2), α-Fluoro-β-alanine (FBAL), Dihydropyrimidine dehydrogenase (DPD)

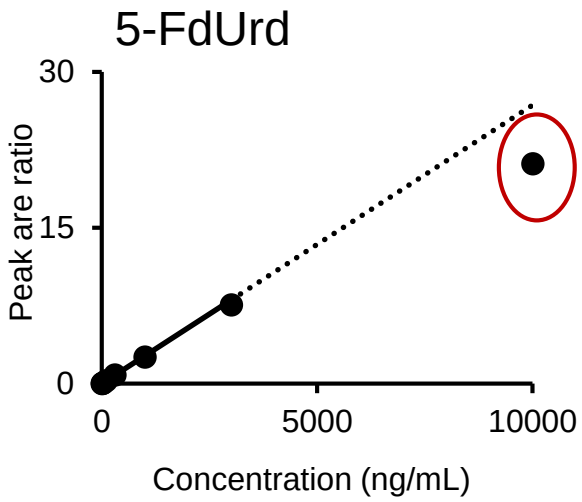
Confirmation of linear range of each analyte

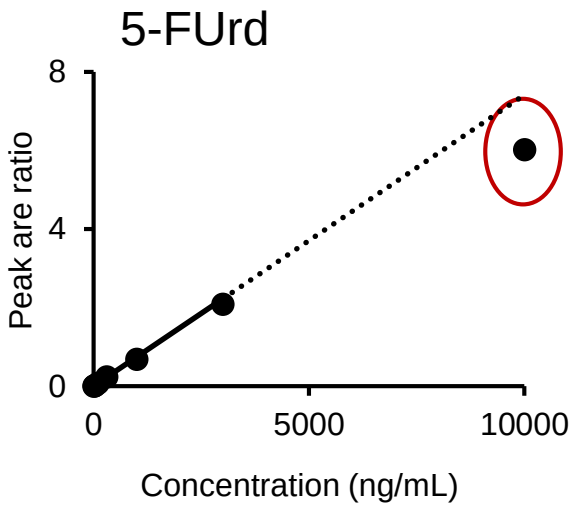




Linear range：10～10,000 ng/mL



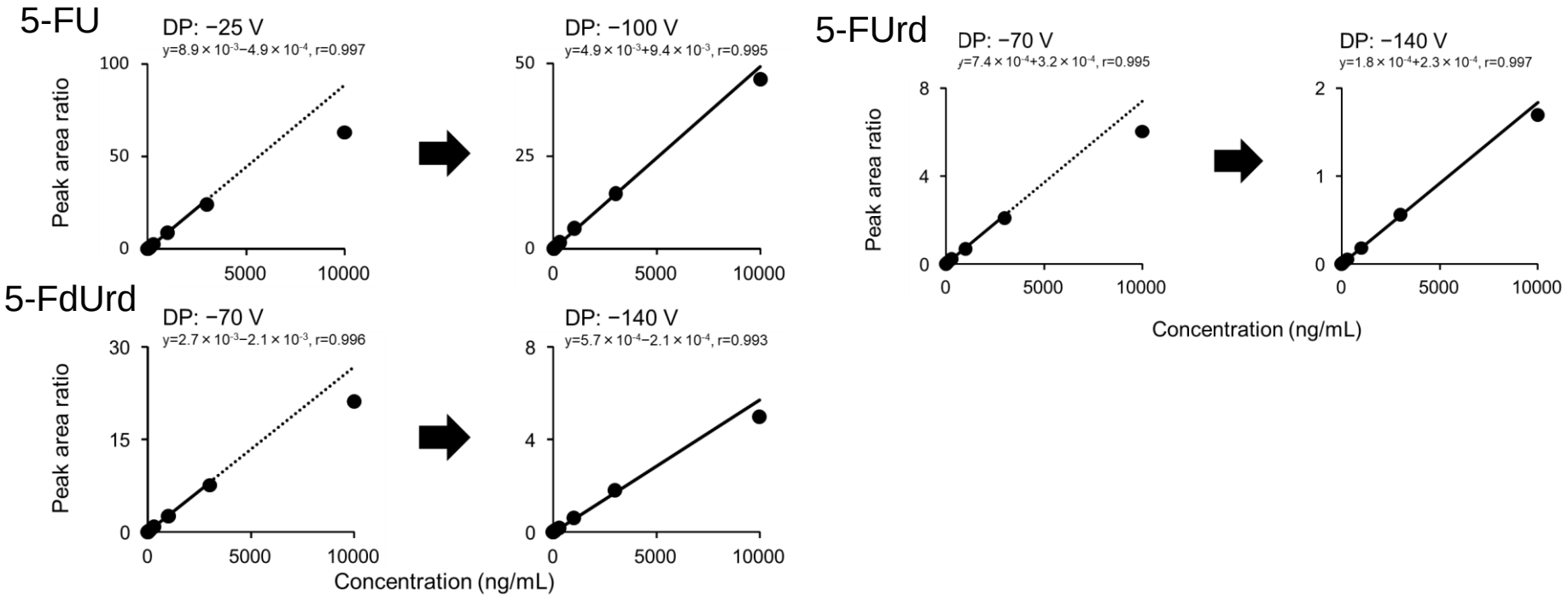




Linear range ：10～3,000 ng/mL

Ishii H et al. *Biomed Chromatogr*. (2016)

Changes of linear range by in-source CID



DP (V)	5-FU (ng/mL)	5-FdUrd (ng/mL)	5-FUrd (ng/mL)
Initial value	3-3,000	3-3,000	3-3,000
Initial value and 70V more negative	3-10,000	3-10,000	10-10,000

It was possible to shift the linear range to 10,000 ng/mL when additional DP of 70 V more negative was applied.

Ishii H et al. *Biomed Chromatogr*. (2016)

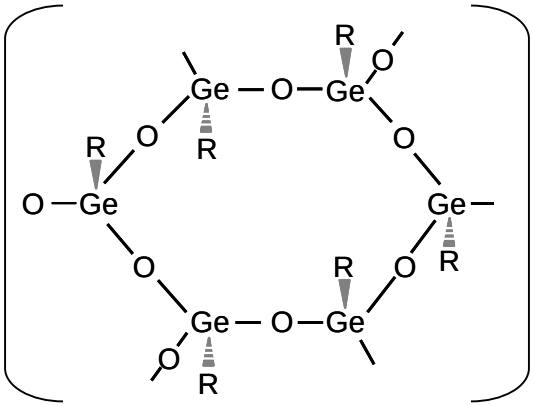
Summary of validation results

	Condition	Results
Linearity	5-FU：3-10,000 ng/mL metabolite：10-10,000 ng/mL	r > 0.99 Accuracy ≦ ±14.1%
Intra-assay	5-FU：3, 10, 300, and 8,000 ng/mL metabolite：10, 30, 300, and 8,000 ng/mL (n=6)	Accuracy ≦ ±11.8% CV ≦ 10.4%
Inter-assay	3 days of intra-assay	Accuracy ≦ ±10.8% CV ≦ 9.9%
Recovery	5-FU：10, 300, and 8,000 ng/mL metabolite：30, 300, and 8,000 ng/mL (n=3)	95.4-111.4%
Matrix effect	5-FU：10 and 8,000 ng/mL metabolite：30 and 8,000 ng/mL 6 individuals（3 males, 3 females）	Accuracy ≦ 12.8% CV ≦ 8.7%

Ishii H et al. *Biomed Chromatogr*. (2016)

The usefulness of the controlling ion amounts by in-source CID for simultaneous determination of compounds with markedly different physicochemical properties in complex biological samples was indicated.

Organic germanium compound Ge-132

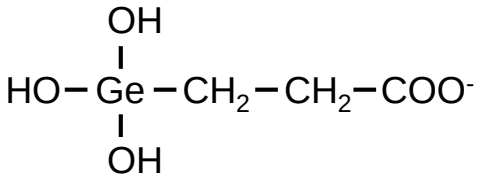


1/3 n

R = CH_2CH_2COOH

$(GeCH_2CH_2COOH)_2nO_3n$

Ge-132



THGP

Ge-132 (Poly-*trans*-[(2-carboxyethyl) germasesquioxane])

Ge-132 is the most common water-soluble organic germanium compound. This compound is hydrolyzed to 3-(trihydroxygermyl)propanoate (THGP) in water.

Physiological effect of THGP

Immunomodulating effect

- Nakamura et al. *Biosci Biotechnol Biochem*. (2012)
- Nakamura et al. *Int J for Vit Nutr Res.* (2014)

Anti-inframatory effect

- Aso et al. *J Biol Response Modif.* (1989)

Analgesic effect

- Suzuki et al., *Ouyouyakuri*, **26:**803-810 (1983)
- Nakamura et al. *Future MedChem*. (2015)

The reason why THGP causes these physiological effects remains unclarified.

We needed information about the structure and the concentration of THGP in these tissues to understand the physiological effects that are caused by THGP.

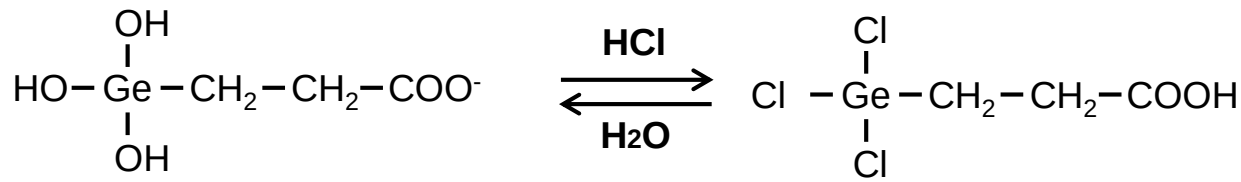
Quantification of germanium compound

- Atomic Absorption Spectrometry (AAS)
- Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

However, these methods measure Ge atom!
It is important to determine intact molecule.

→ We developed the quantification method of intact THGP by LC/ESI-MS/MS for detailed THGP pharmacokinetic analysis.

A novel extraction method based on a reversible chemical conversion



3-(Trihydroxygermyl)propanoate

THGP

3-(Trichlorogermyl)propanoic acid

TCGPA

100 µL plasma

↓ IS (Ge-132-*d2*)

↓ HCl 200 µL

↓ Chloroform 2 mL

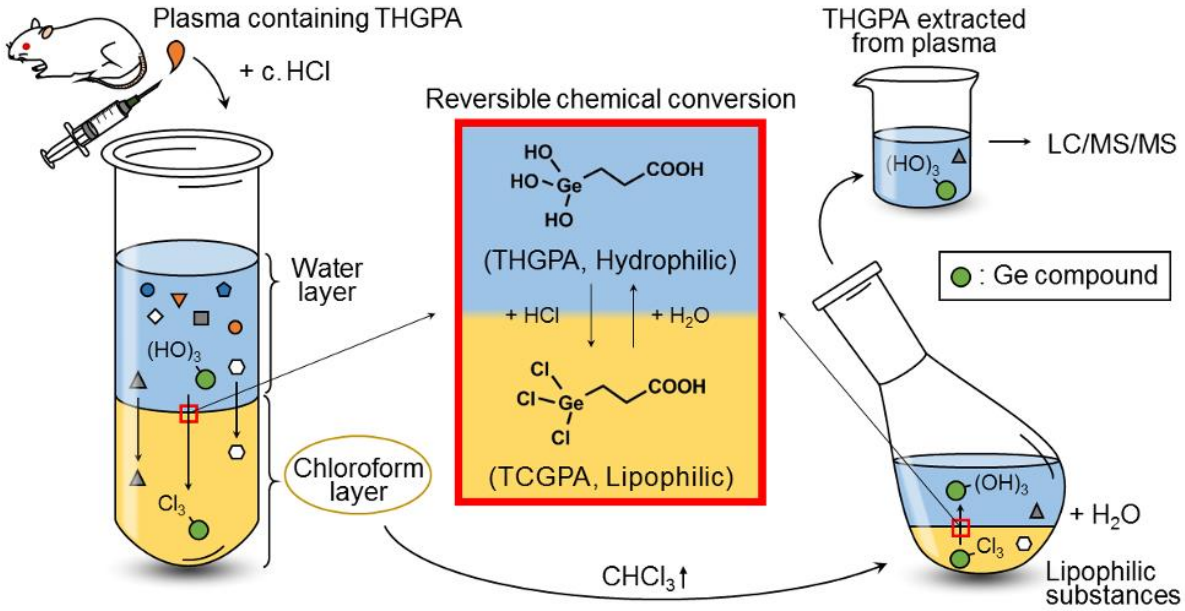
↓ Incubate for 1 hr

↓ Collection chloroform layer

↓ Evaporation

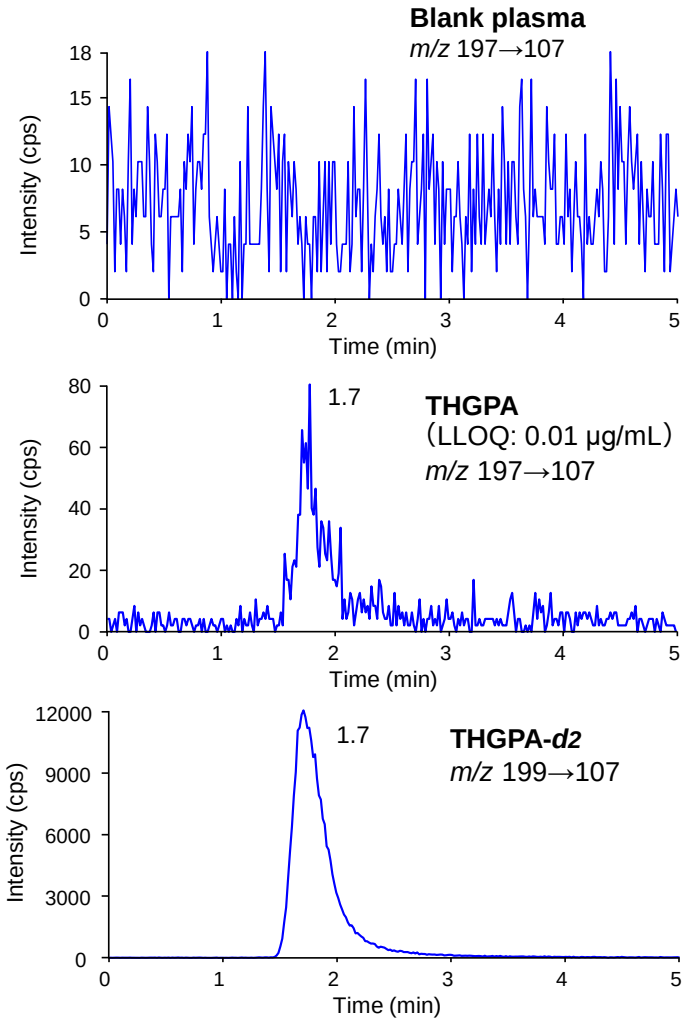
↓ 50 µL watar

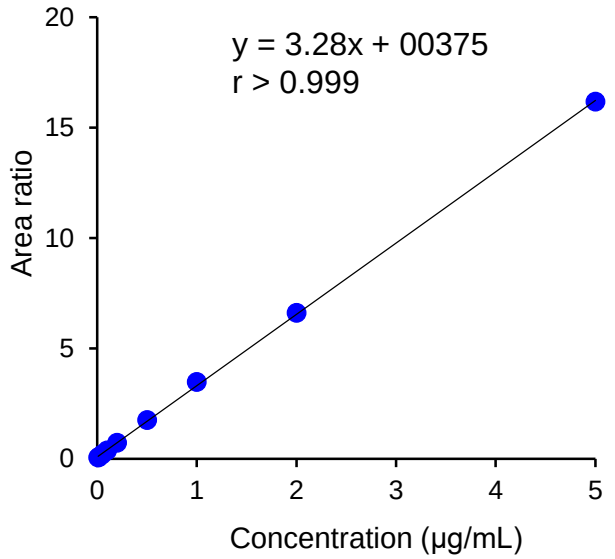
↓ 5 µL injection into LC/MS/MS



Yamaguchi H et al. *Anal Chem.* (2015)

A novel extraction method based on a reversible chemical conversion





Analytical range 0.01 – 5 μg/mL (as Ge-132)

Chen et al.（HPIEC）[1)]
Ge-132： 0.1 – 100 μg/mL
Trikas et al.（GC-MIP-AED）[2)]
Ge-132： 1 – 250 μg/mL

1. Chen et al. *J Chromatogr A.* (1997)
2. Trikas et al. *Anal Bioanal Chem.* (2014)

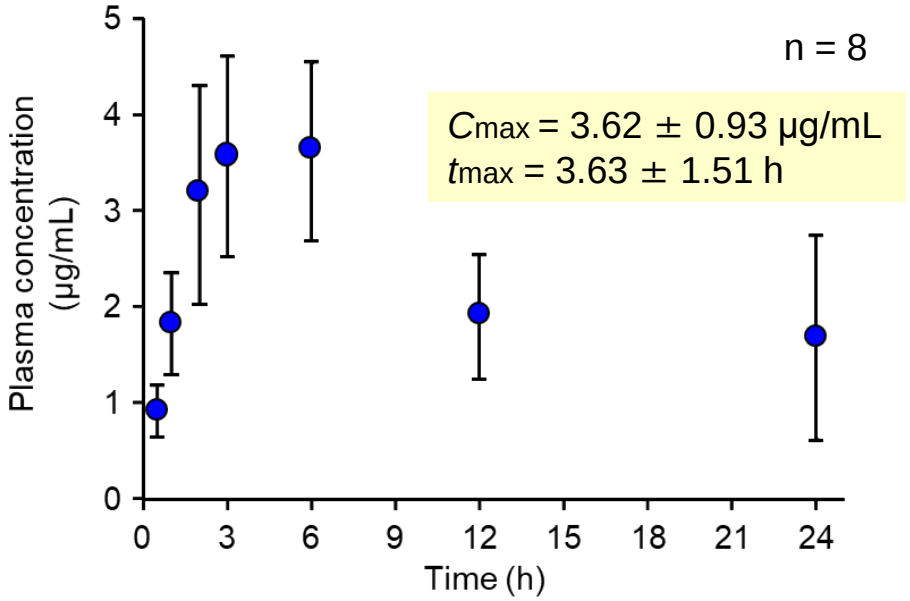
Analytical column RSpak DE413-2D
(150 mm x 2.0 mm i.d., Showa Denko)
Mobile phase Water-methanol-acetic acid (95:5:0.1, v/v/v)
Flow rate 300 μL/min Column temperature 50°C

10-100 times higher sensitivity
compared to previous reports

Yamaguchi H et al. *Anal Chem.* (2015)

Application to a pharmacokinetic study

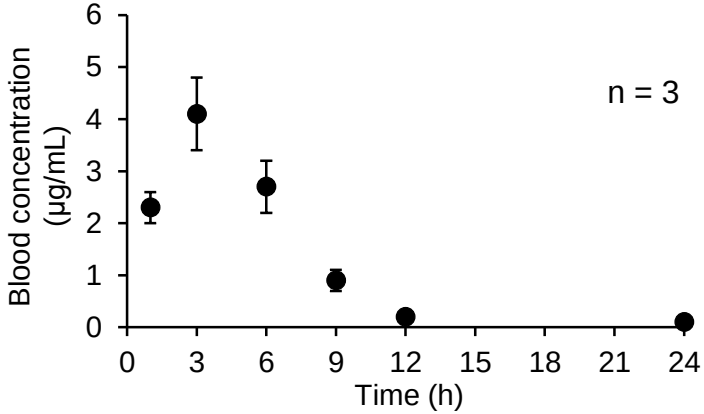
Wistar rat♂ fasted overnight
Ge-132 100 mg/kg p.o.



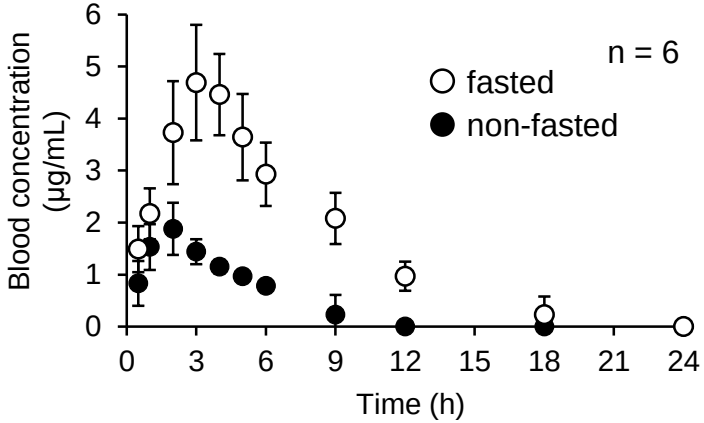
The value of our study (C_{max} 3.62 μg/mL) was concentration in plasma, and therefore the theoretical blood concentration is 2.26 μg/mL (calculated as hematocrit value 50% and 80% distribution in plasma).
Compared to the previous report, C_{max} was low, but AUC was comparable.
The agreement among these three analytical methods suggests that almost all of the THGP remains without metabolism in blood.

Yamaguchi H et al. *Anal Chem.* (2015)

Wistar rat♂ fasted for 24 hr
^{14}C-Ge-132 100 mg/kg p.o.[1)]



Wistar rat♂
Ge-132 100 mg/kg p.o.[2)]
Determined by AAS



1. Kagoshima et al. *Ouyouyakuri.* (1986)
2. Kagoshima et al. Asai gerumanium in-house document

Application of LC/ESI-MS/MS methods to the pharmacokinetic studies

Bile acid

Steroids, **78:**967-972 (2013)
PLoS One, **12:**e016719 (2017)
Ann Clin Biochem, **52:**576-587 (2015)
Mass Spectrom (Tokyo), **5:**S0053 (2016)

Prostaglandins

Prostaglandins Leukot Essent Fatty Acids, **76:**321-329 (2007)
J Chromatogr B Analyt Technol Biomed Life Sci, **879:**3378-3385 (2011)
Prostaglandins Other Lipid Mediat, **106:**37-44 (2013)
Prostaglandins Leukot Essent Fatty Acids, **91:**61-71 (2014)
PLoS One, **9:**e109270 (2014)
Anal Bioanal Chem, **407:**1625-1639 (2015)

OATP substrates

J Chromatogr B Analyt Technol Biomed Life Sci, **972:**73-80 (2014)
J Pharm Pharm Sci, **17:**475-484 (2014)
J Pharmacol Exp Ther, **362:**271-277 (2017)

Anti-cancer drugs

J Chromatogr B Analyt Technol Biomed Life Sci, **893-894:**157-161 (2012)
J Pharm Biomed Anal, **71:**99-103 (2012)
Biomed Chromatogr, **27:**539-544 (2013)
J Chromatogr B Analyt Technol Biomed Life Sci, **917-918:**18-23 (2013)
Chromatography, **38:**95-100 (2017)
Biomed Chromatogr, **32:**e4184 (2018)
Chromatography, **39:**41-47 (2018)

Immunosuppressive drugs

J Chromatogr B Analyt Technol Biomed Life Sci, **879:**968-974 (2011)
J Chromatogr B Analyt Technol Biomed Life Sci, **879:**987-992 (2011)
Ther Drug Monit, **39:**648-653 (2017)
J Pharm Health Care Sci, **4:**7 (2018)

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