The Use of SCAP for the Quantitative Bioanalysis of Drugs

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DBS card

FTA DMPK Card Selection

Quoted by Whatman® HP

A

FTA DMPK-A

- Blood spots dry within 2 h
- Blood spot area is ~20% smaller than DMPK-B or DMPK-C cards
- Protein denaturing activity will inactivate endogenous enzymes
- Cell lysis releases endogenous cellular materials onto card
- Stabilization of DNA allows resampling of blood spot for pharmacogenomics
- Impregnated chemicals may interfere with mass spectrometry detection e.g. ion suppresion

B

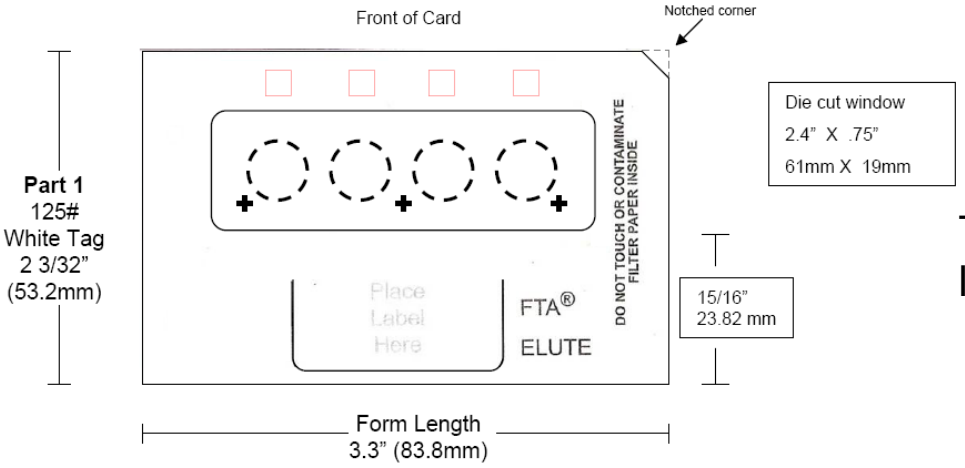
FTA DMPK-B

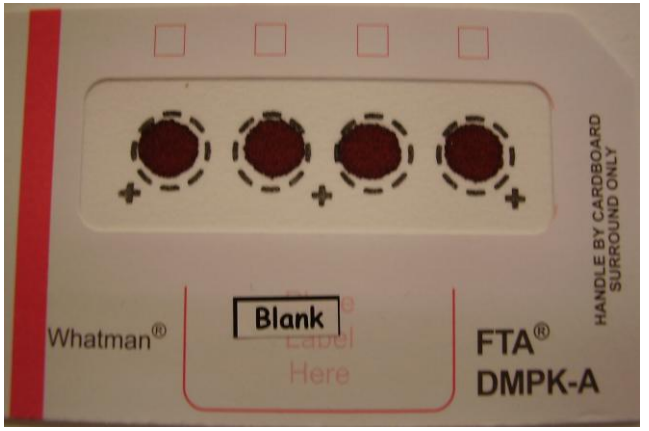
- Blood spots dry within 2 h
- Protein denaturing activity will inactivate endogenous enzymes
- Cell lysis releases endogenous cellular materials onto card
- Stabilization of DNA allows resampling of blood spot for pharmacogenomics
- Impregnated chemicals may interfere with mass spectrometry detection e.g. ion suppresion

C

FTA DMPK-C

- Blood spots dry within 2 h
- No impregnated chemicals to interfere with analysis
- Proteins are not denatured so cards may be better suited for protein based biomolecules





After whole blood sample spotting on DBS card

The FTA® DMPK-A and FTA ® DMPK-B cards lyse cells and denature proteins on contact.

- **FTA DMPK-A Card**
- **FTA DMPK-B Card**
- **FTA DMPK-C Card**

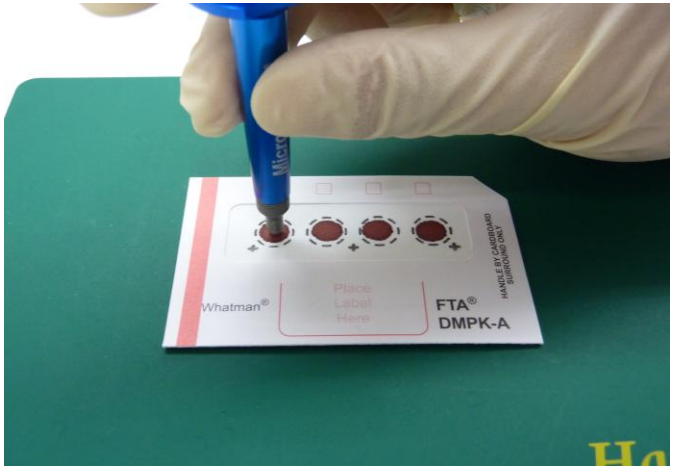
Advantages

- Reduced sample volume (typically 10 to 20 µL)
  - Blood could be collected from one small animal (e.g. rodent) for multiple sampling points
  - Removal of satellite rodent group
  - Data quality would be increased (e.g. TK parameters)
  - Enables juvenile studies
  - For animal care

- Efficient sample processing
  - Small number of animals
    （satellite group is not required)
  - Simplified to collect study sample
    (separation of plasma from blood is unnecessary)

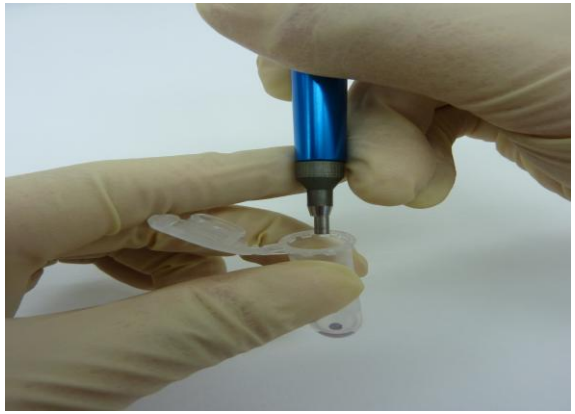
- Reduced costs
  - Transportation (Dry ice is not required)
  - Detoxification (inactivation of HIV and Hepatitis B and C)
  - Storage condition
    (Basically DBS cards are stored under room temperature.)

DBS (Offline)



Punch out sample disc

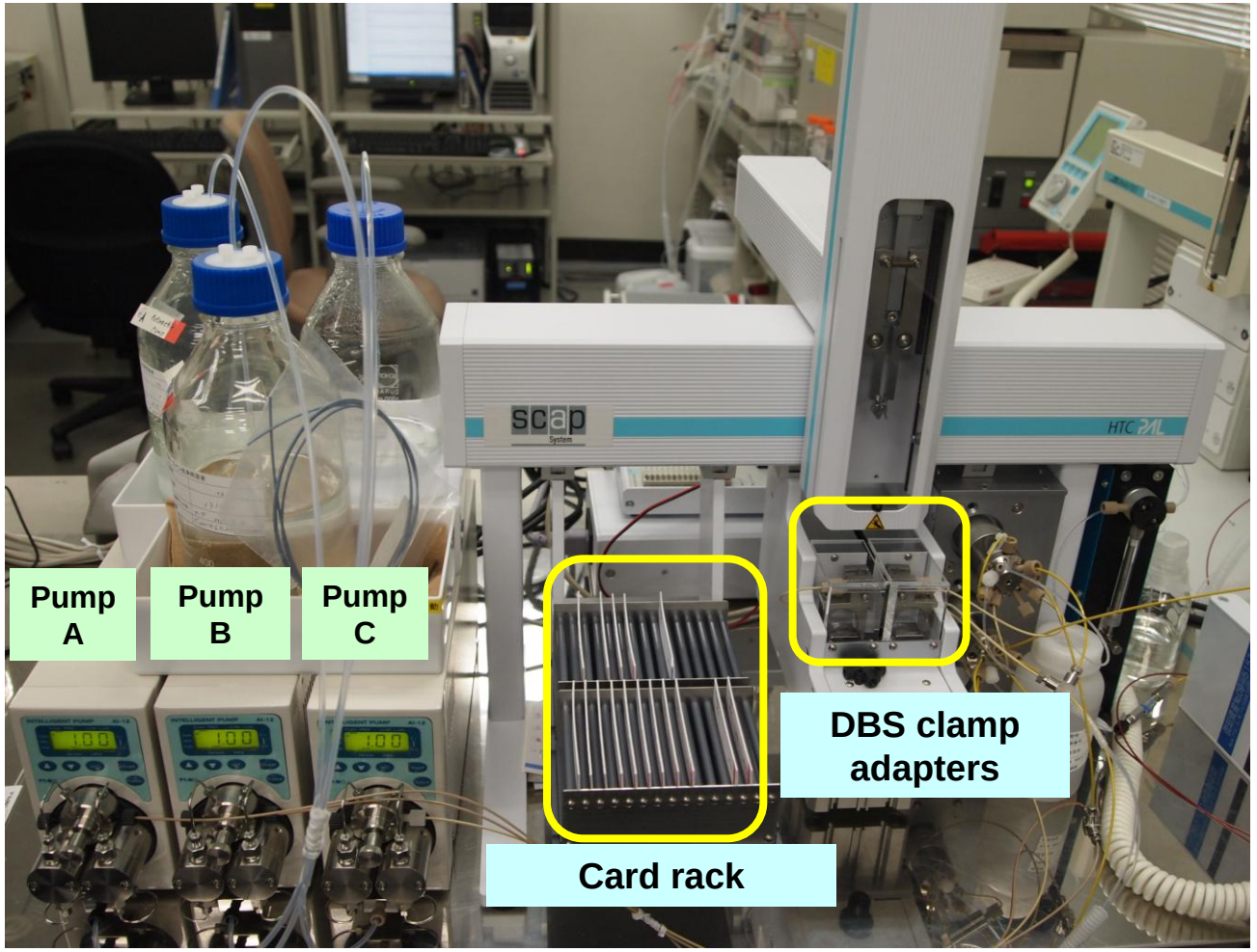
LC/MS/MS analysis



Pretreatment

SCAP system

SCAP (Sample Card & Prep)



LC/MS/MS analysis

Benefit of SCAP

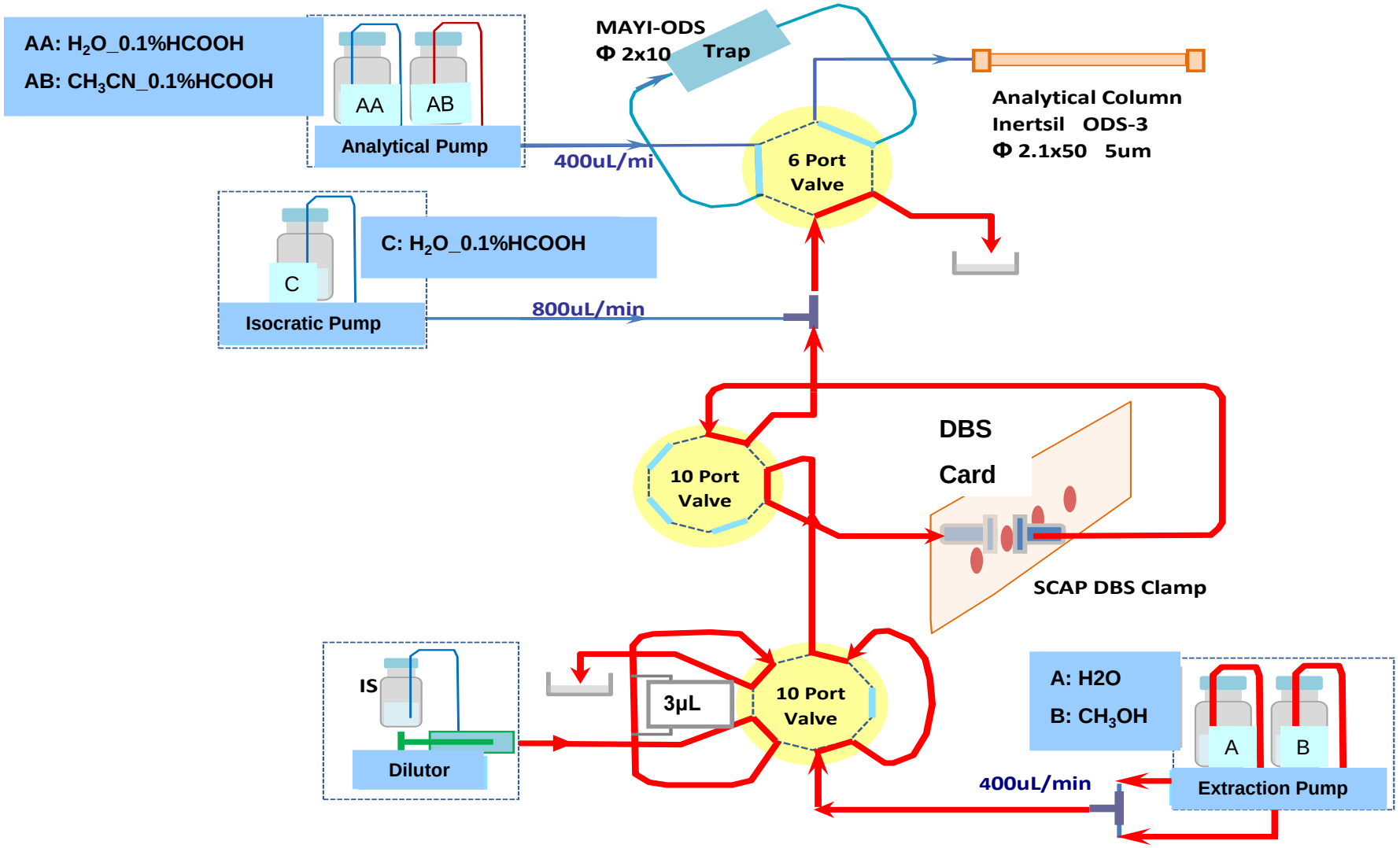
- **High data quality**
  Improvement of reproducibility due to automatic operation by SCAP system

- **Efficient sample processing**
  Productivity of acquisition data would be increased

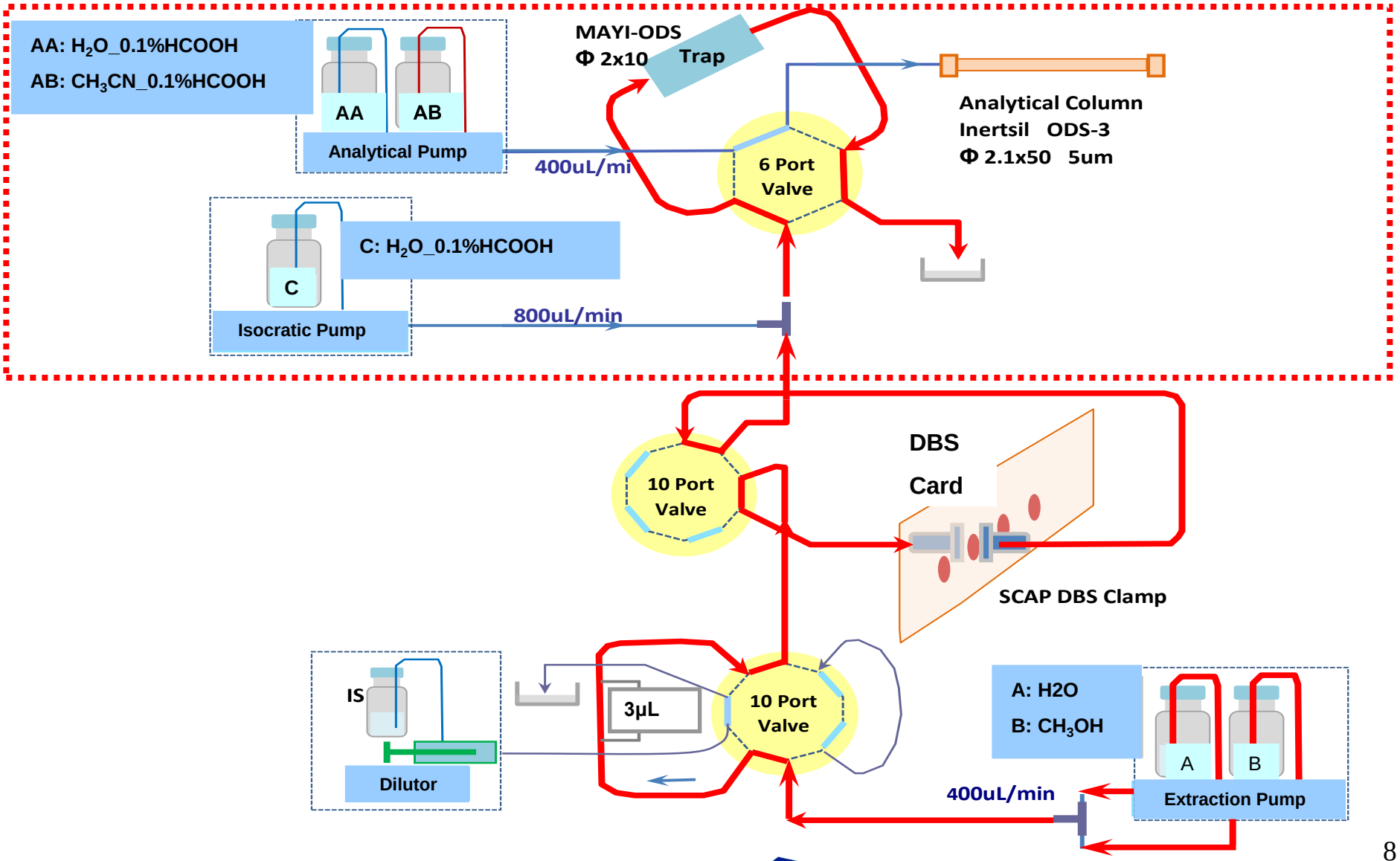
- **High sensitivity measurement**
  Using enrichment column for online pretreatment leads to higher sensitivity

- **Reduction of biohazard risk**
  Safety assurance for researcher/personnel

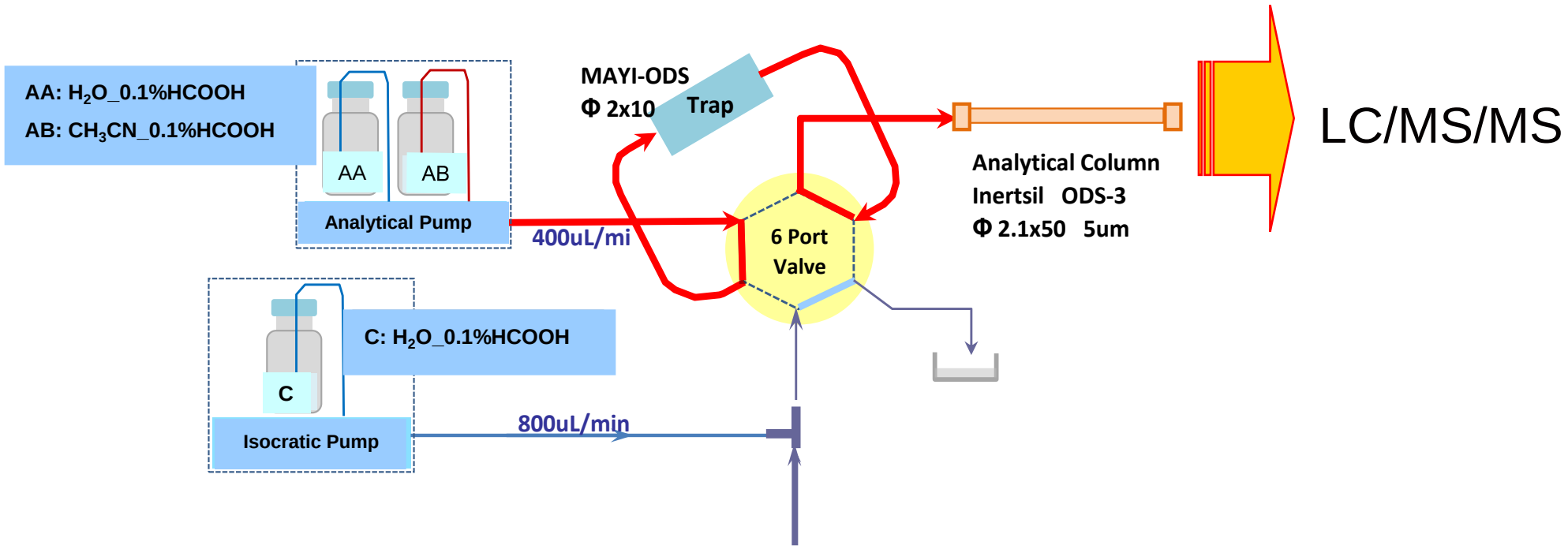
Flow channel (Card cleaning)



Flow channel (Enrichment)



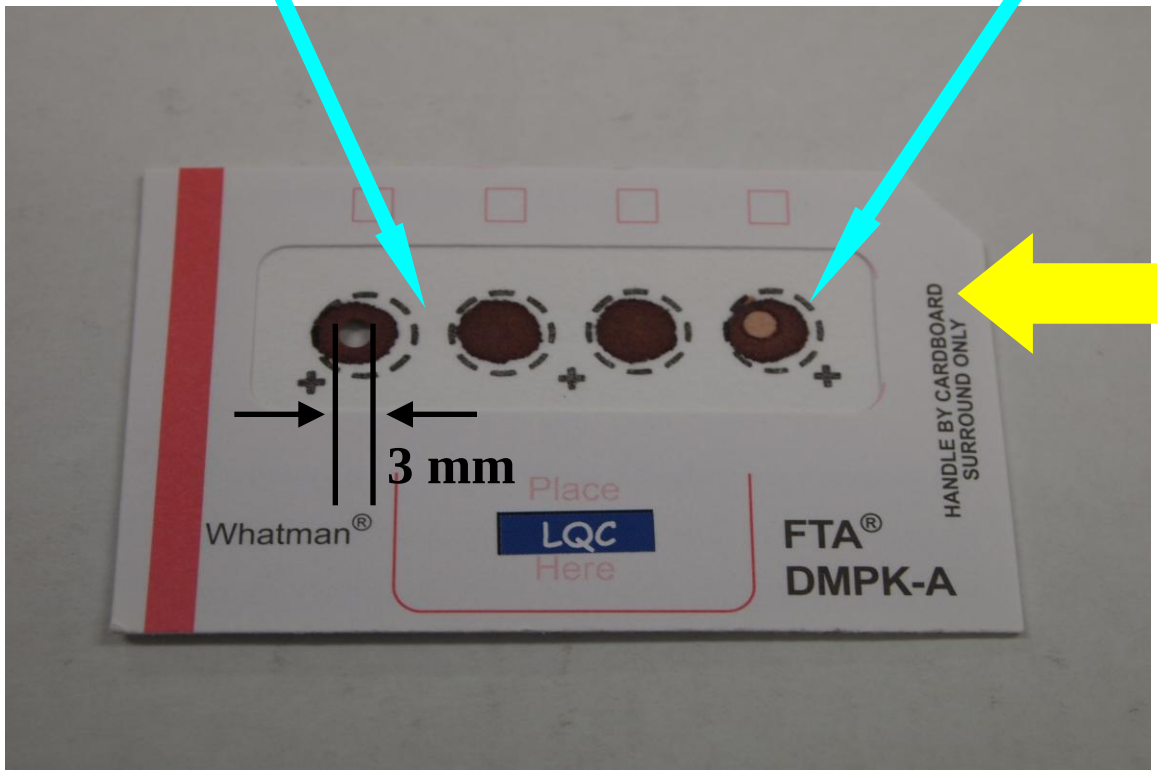
Flow channel (Analysis)



DBS card

Punch out sample disc
(offline pretreatment)

Extraction using SCAP



State of card after
treating extraction

Circle sizes from each treatment method were almost equal.

Experiments

SCAP performance was evaluated as listed below.

➢Screening

Availability of DBS HTS method for sartan and statin drugs
Stability of analyte on DBS card

➢Quantification

Calibration standards and QC samples

Why is HTS method required?

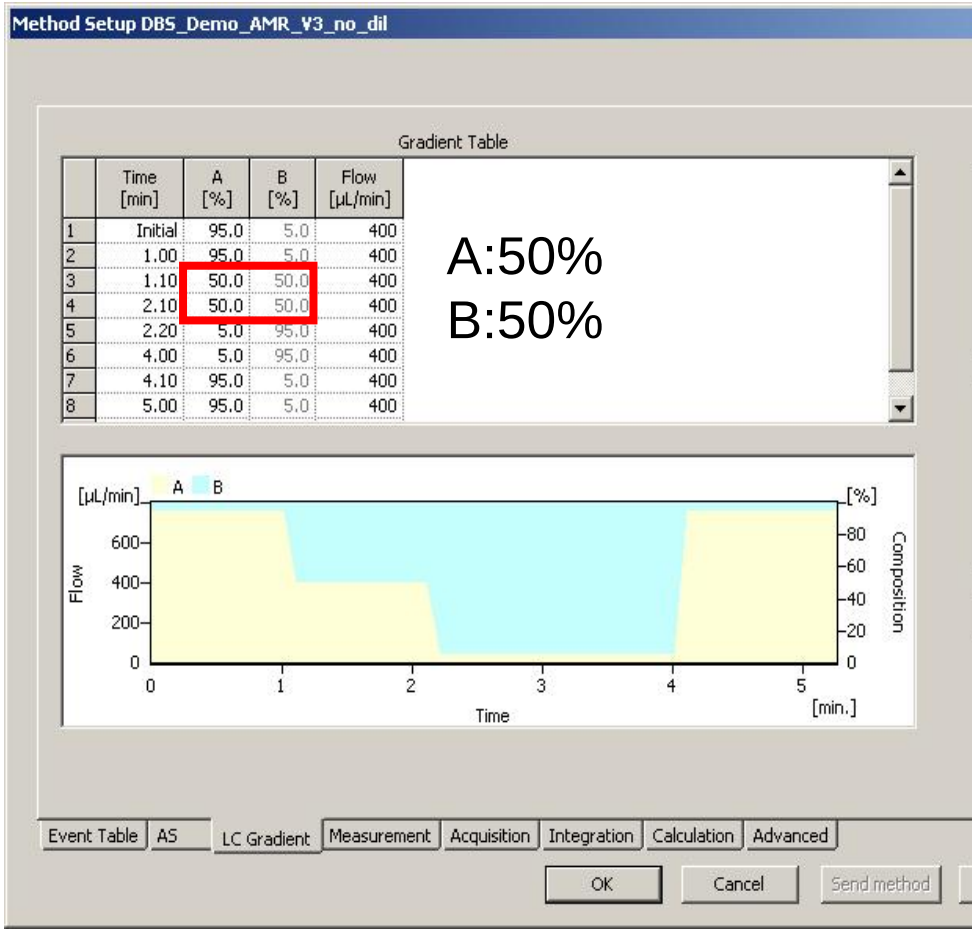
Analyte, which is not suitable for chemically-coated DBS card, can not be measured.

e.g.) denaturation ?, degradation ? …

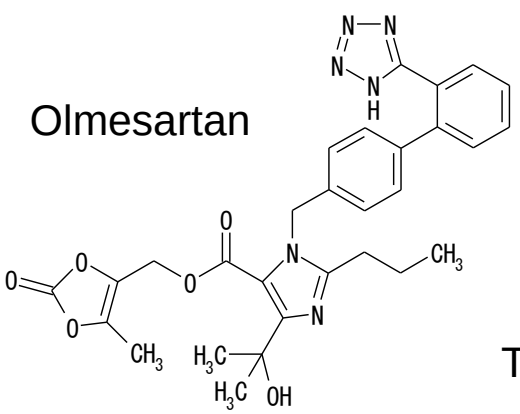
Preliminary test should be performed prior to conduction for method validation.

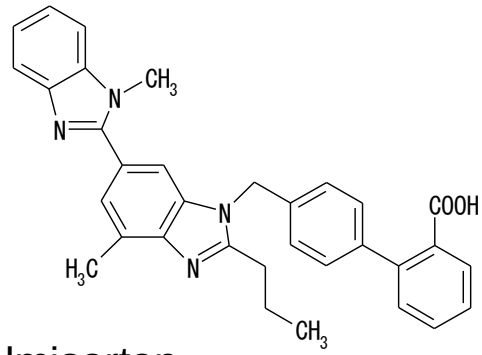
Development of high throughput screening method would be required.

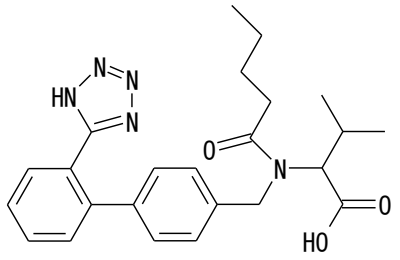
Extraction condition

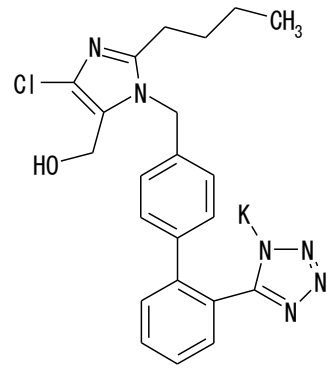


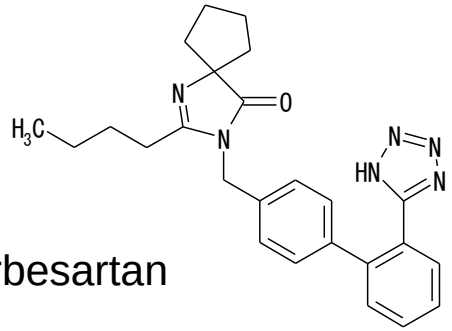
Under the same extraction conditions for 5 sartan drugs.



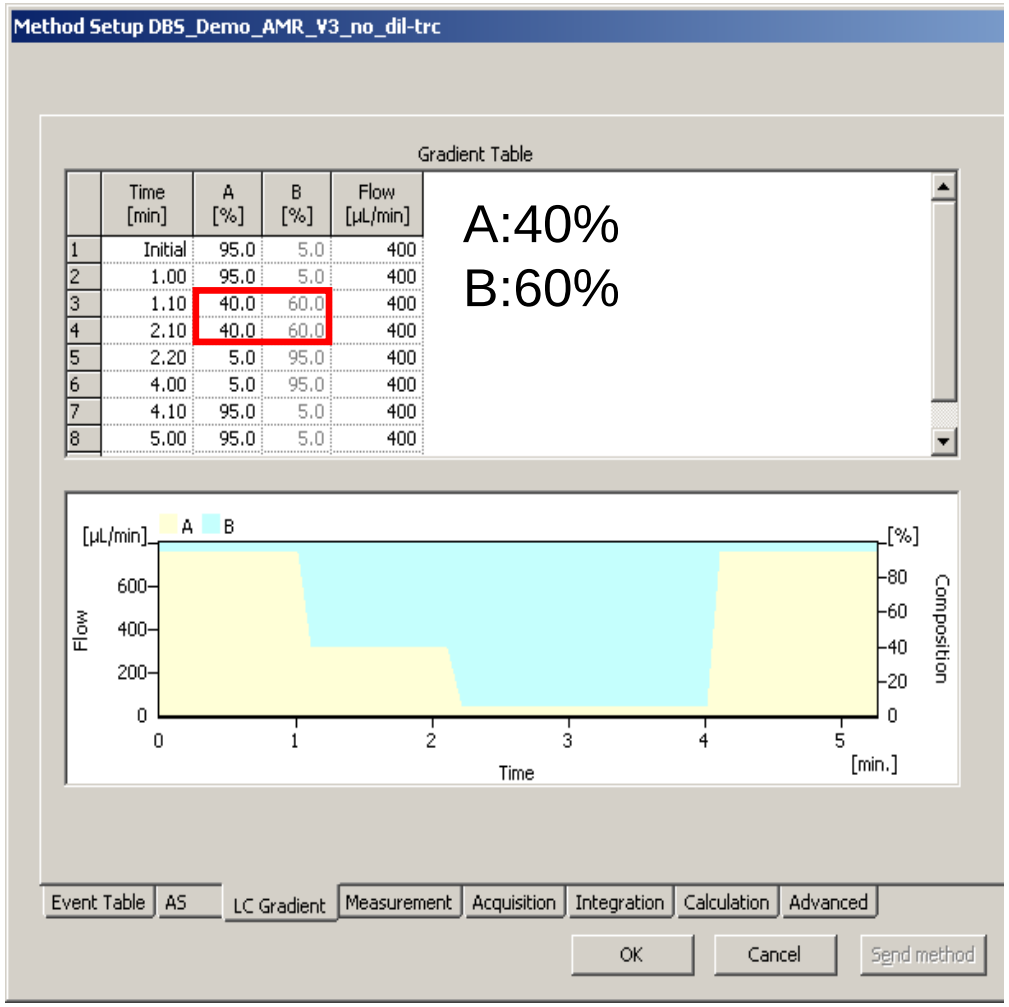


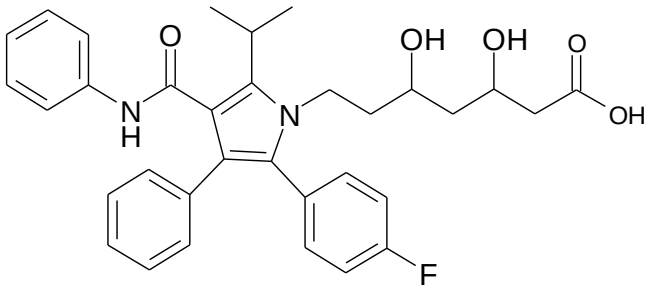




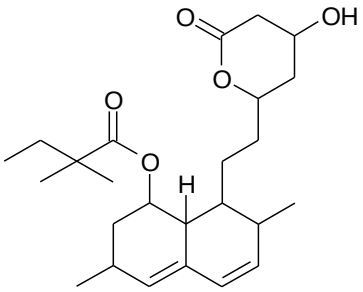


Extraction condition

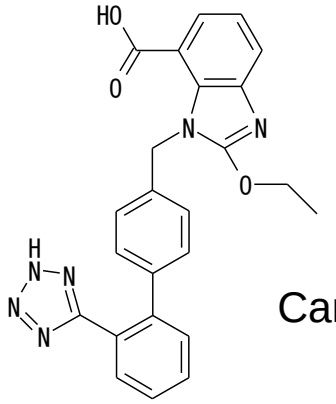




Atorvastatin



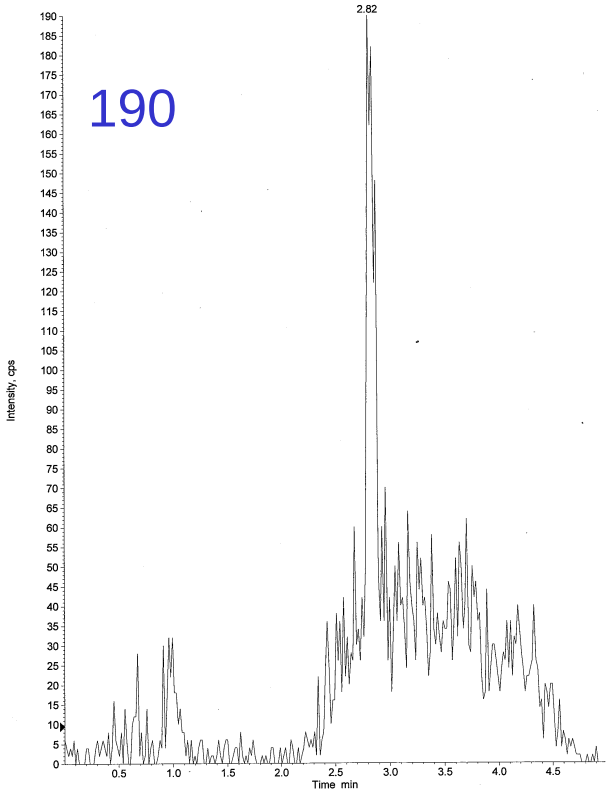
Simvastatin



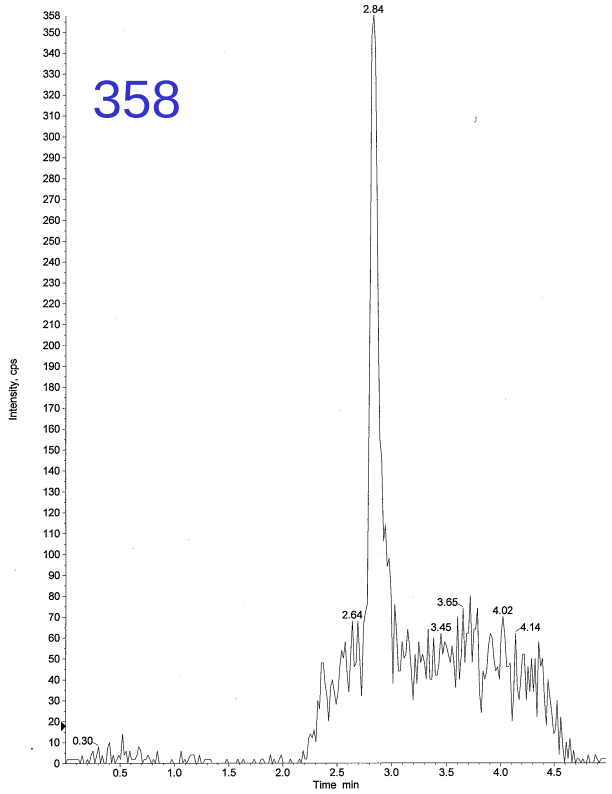
Candesartan

Chromatograms of Valsartan (10 ng/mL)

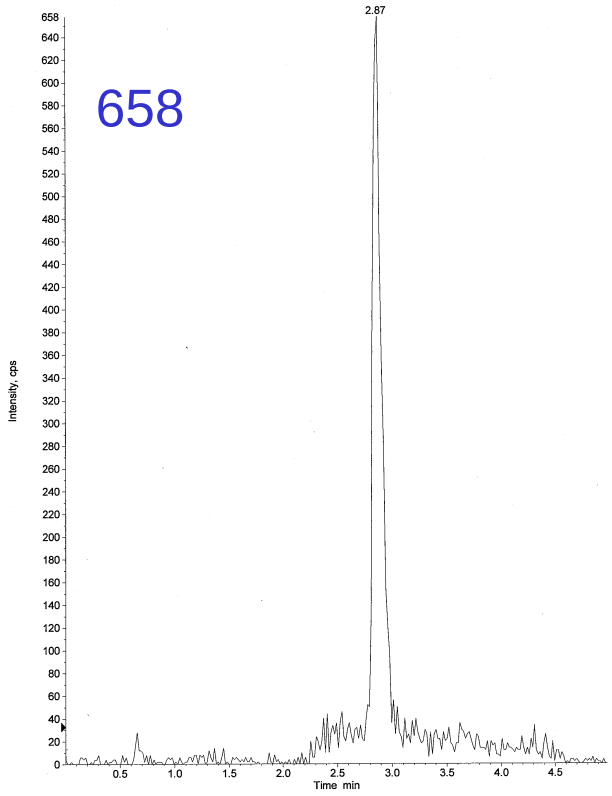
A Card



B Card

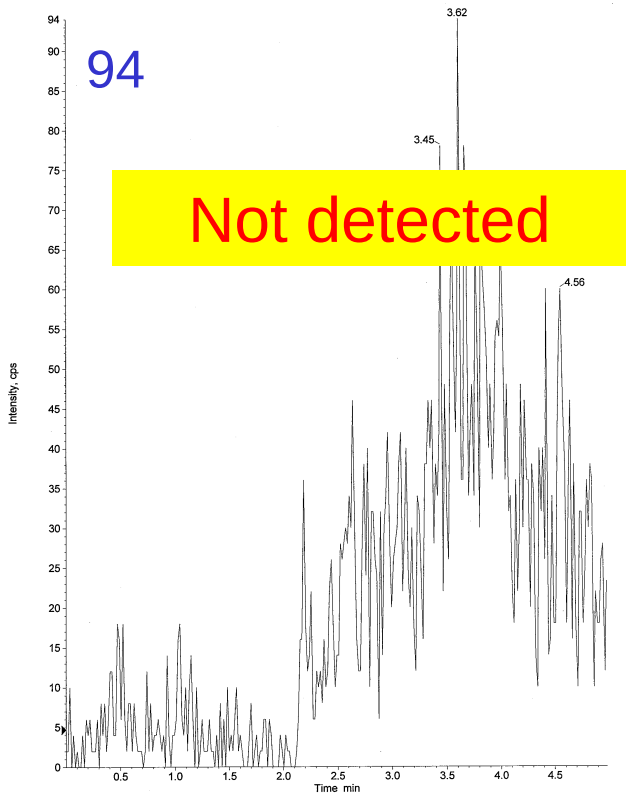


C Card

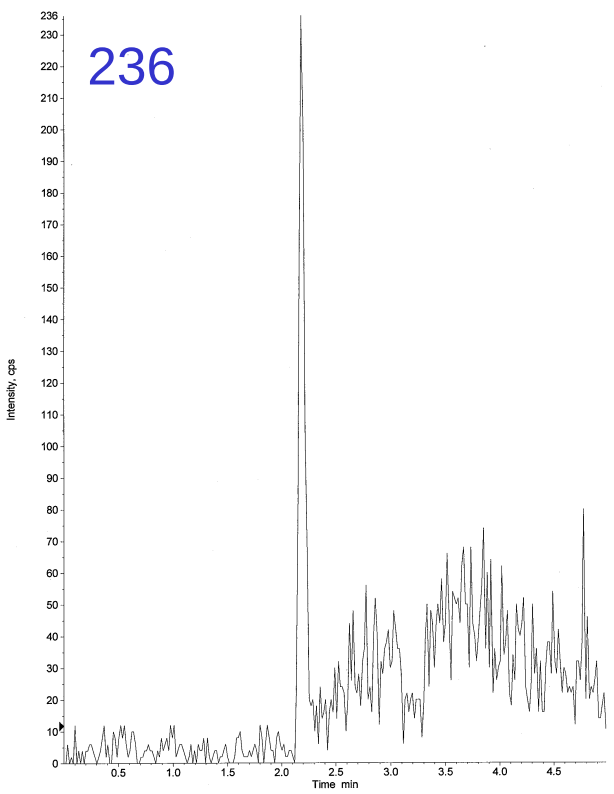


Chromatograms of Olmesartan (10 ng/mL)

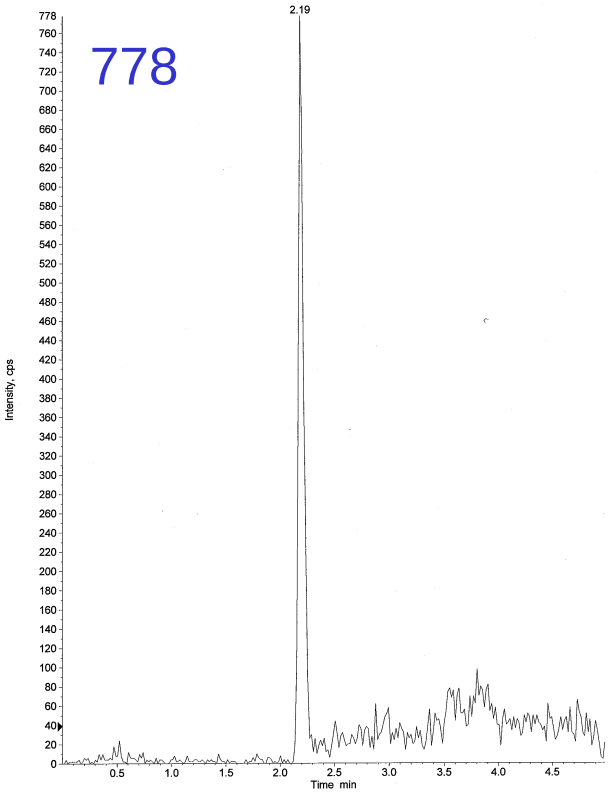
A Card



B Card

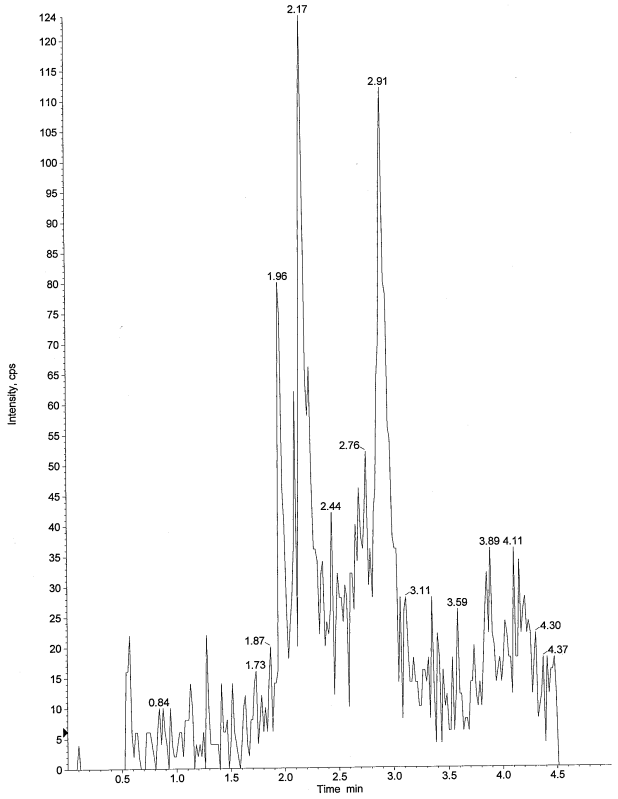


C Card

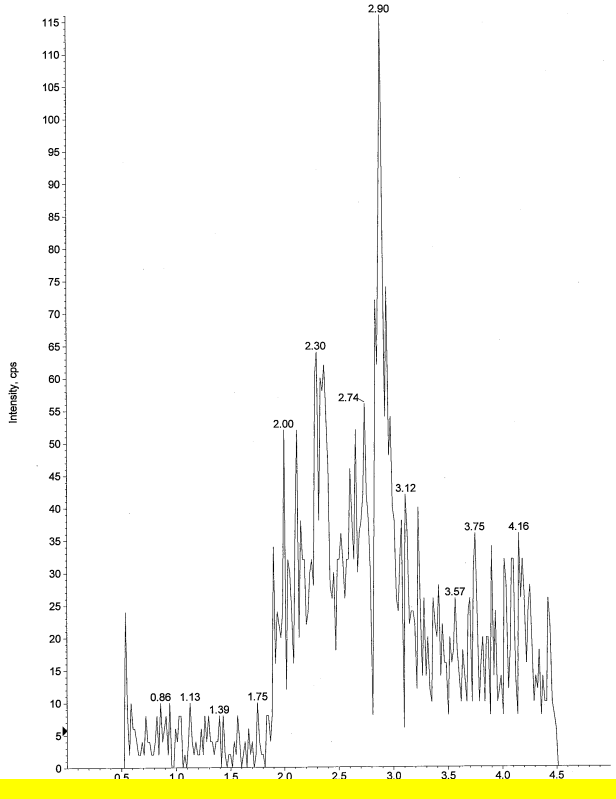


Chromatograms of Simvastatin (10 ng/mL)

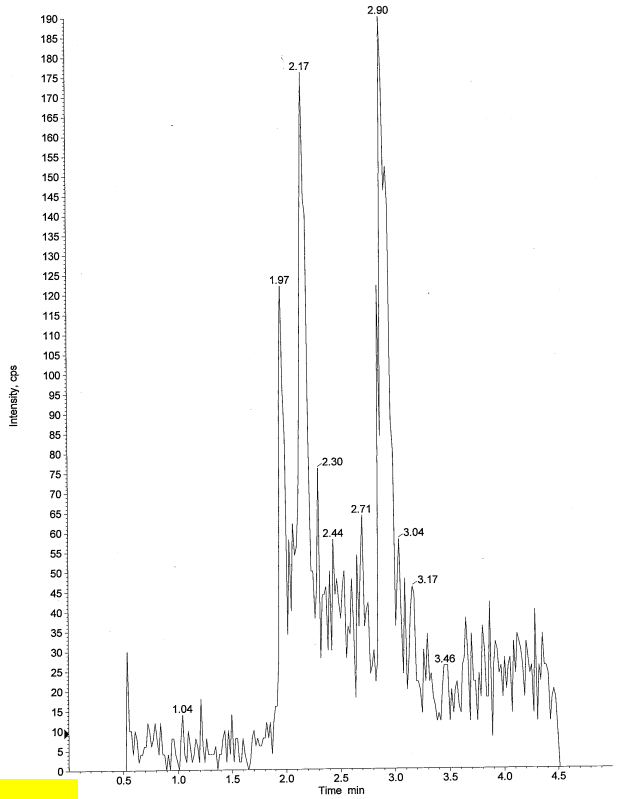
A Card



B Card



C Card



Not detected

Suitable for DBS card?

Suitability for each DBS card, on which 10 ng/mL working solution was spotted, was judged based on the result of peak intensity obtained by the analysis of extracted analyte from the card.

Compound name	A card	B card	C card
Valsartan	△	△	◎
Telmisartan	○	○	◎
Olmesartan	×	△	◎
Irbesartan	○	○	◎
Losartan	○	◎	○
Candesartan	○	◎	○

× : Not detected
△ : S/N >3
○ : Good
◎ : Strongest intensity

Suitable for DBS card?

Suitability for each DBS card, on which 10 ng/mL working solution was spotted, was judged based on the result of peak intensity obtained by the analysis of extracted analyte from the card.

Compound name	A card	B card	C card
Atorvastatin	○	◎	△
Simvastatin	×	×	×

× : Not detected
△ : S/N >3
○ : Good
◎ : Strongest intensity

Treatment capacity (Offline)

Time required per 1 compound

Conditions

Working solution: 2 levels (low and high concentrated)
DBS card: 3 cards (A, B, and C)

Process	Time required
Weigh and preparation	1 hour
Working solution spot and dry-up	1 hour
Pretreatment	2 hour
LC/MS/MS analysis	1 hour

Total time: 5 hours

8 compounds

Information about the effect of DBS cards on multiple compounds could be obtained for 4 to 8 days.

Treatment capacity (SCAP system)

Time required per 1 compound

Conditions

Working solution: 2 levels (low and high concentrated)
DBS card: 3 cards (A, B, and C)

Process	Time required
Weigh and preparation	1 hour
Working solution spot and dry-up	1 hour
LC/MS/MS analysis	1 hour

8 compounds

Information about the effect of DBS cards on multiple compounds could be obtained for only **1** day.

Total time: 3 hours

Quantitative analysis

Conditions

- Whole blood: Rat
- Spotted on card: 15 µL
- Calibration curve
  10 ng/mL to 10000 ng/mL
- Assay reproducibility (Intra-assay)
  QC samples:3 levels

  (L: 20 ng/mL, M: 400 ng/mL, H: 8000 ng/mL)

Quantitative analysis (Calibration curve)

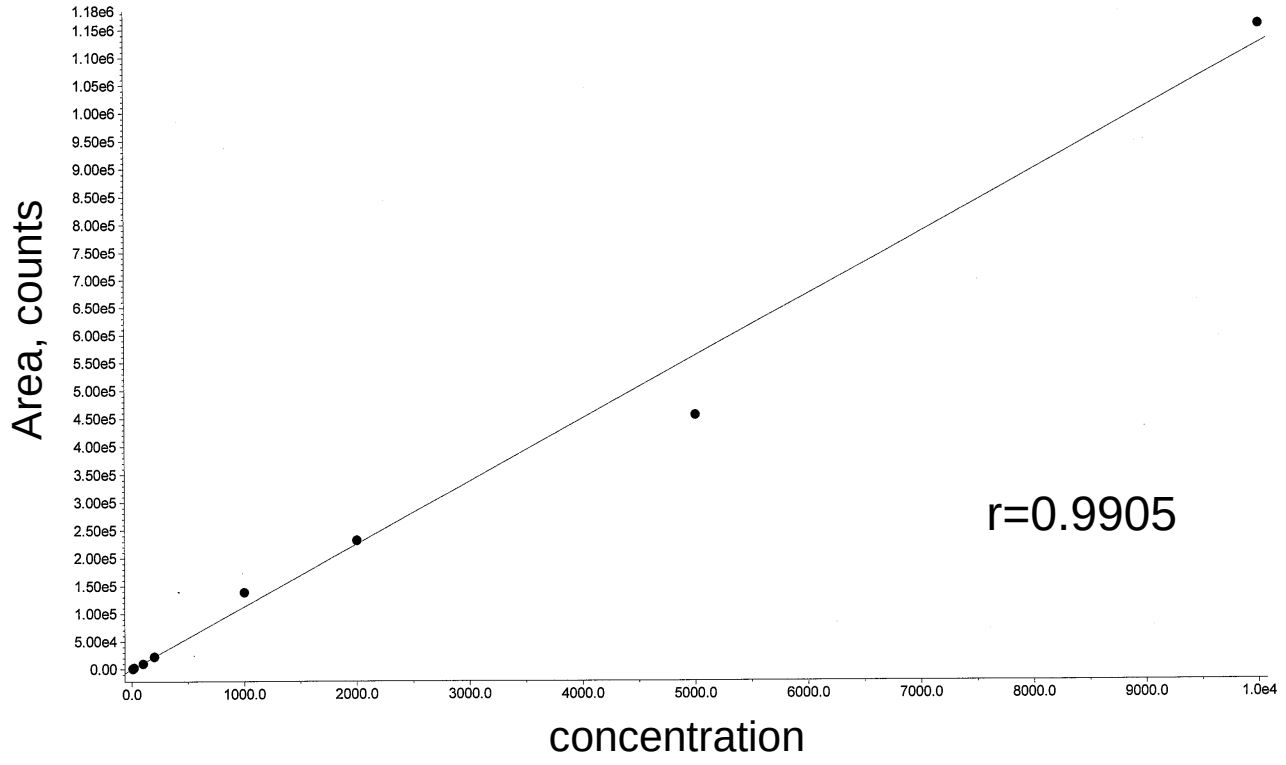
Valsaran concentration (ng/mL)							
10.0	**20.0**	**100**	**200**	**1000**	**2000**	**5000**	**10000**
9.87	21.0	86.6	200	1230	2060	4050	10300
98.7	105.0	86.6	100.0	123.0	103.0	81.0	103.0

Upper value: Found concentration (ng/mL)

Lower value: Accuracy (%Nominal)

Linear regression ($1/x^2$ weighting)

Absolute calibration curve



Quantitative analysis (Intra-assay)

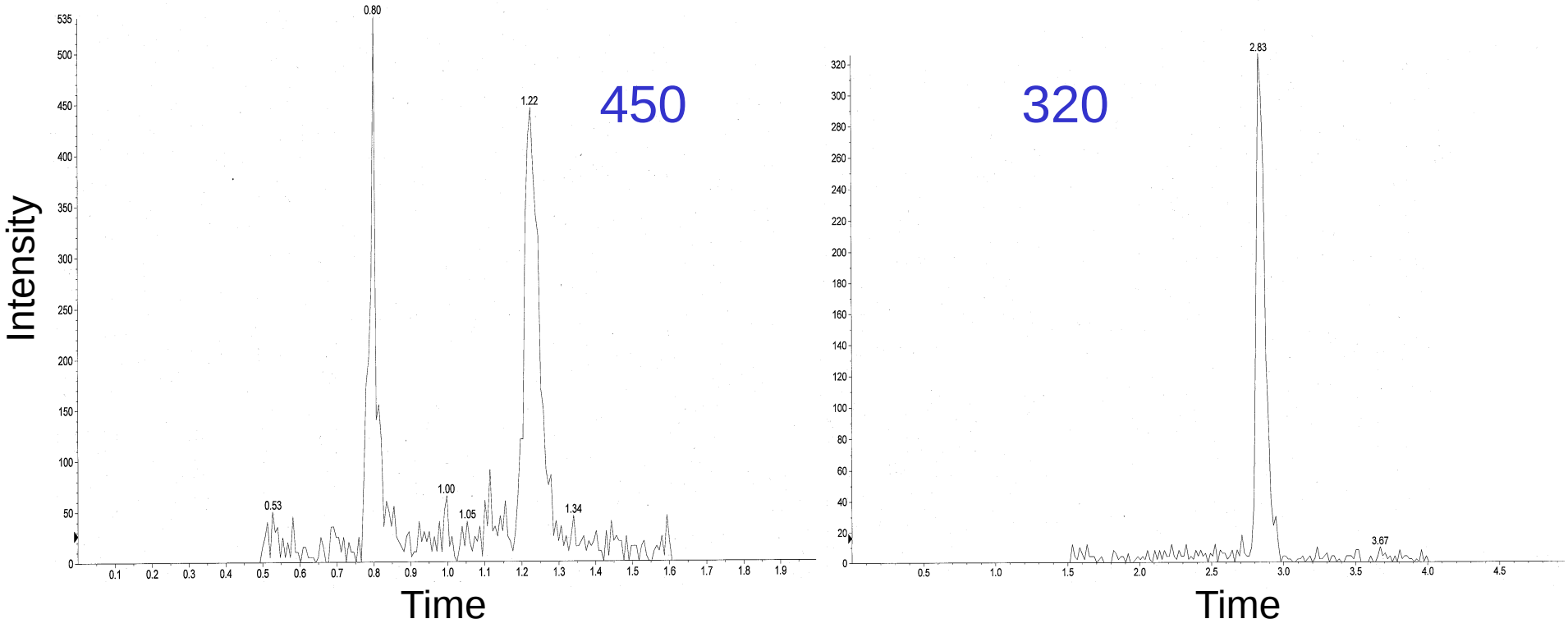
Result of intra-assay reproducibility (N=5)

	Valsartan concentration (ng/mL)		
QC sample	LQC	MQC	HQC
Nominal conc. (ng/mL)	20.0	400	8000
Conc. (ng/mL)	24.3	456	9290
	21.3	420	8300
	26.4	412	9310
	25.7	488	10500
	25.2	399	7780
Mean conc.	24.6	435	9040
SD	2.00	36.4	1049.1
Accuracy (%Nominal)	123.0	108.8	113.0
Precision (%CV)	8.1	8.4	11.6

SCAP vs. Offline

Offline

SCAP



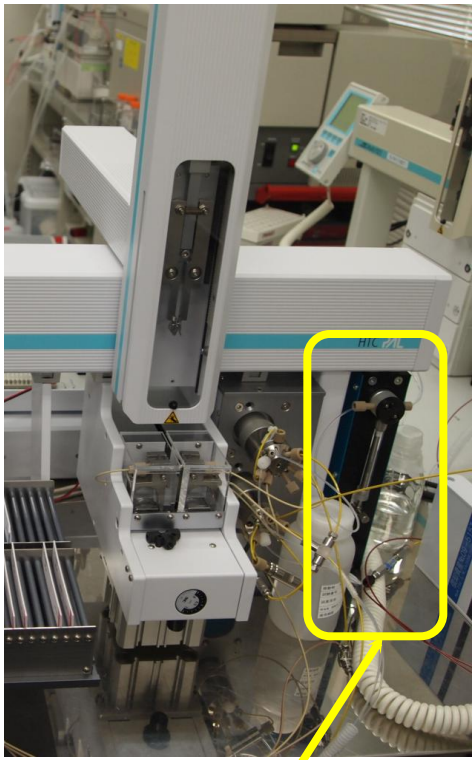
API5000 (AB Sciex)

API4000 (AB Sciex)

Chromatograms of Valsartan (10 ng/mL) from rat whole blood

Approach to GLP study

Evaluation of stability



Dilutor for injecting IS

How can reproducible IS peak areas be acquired?

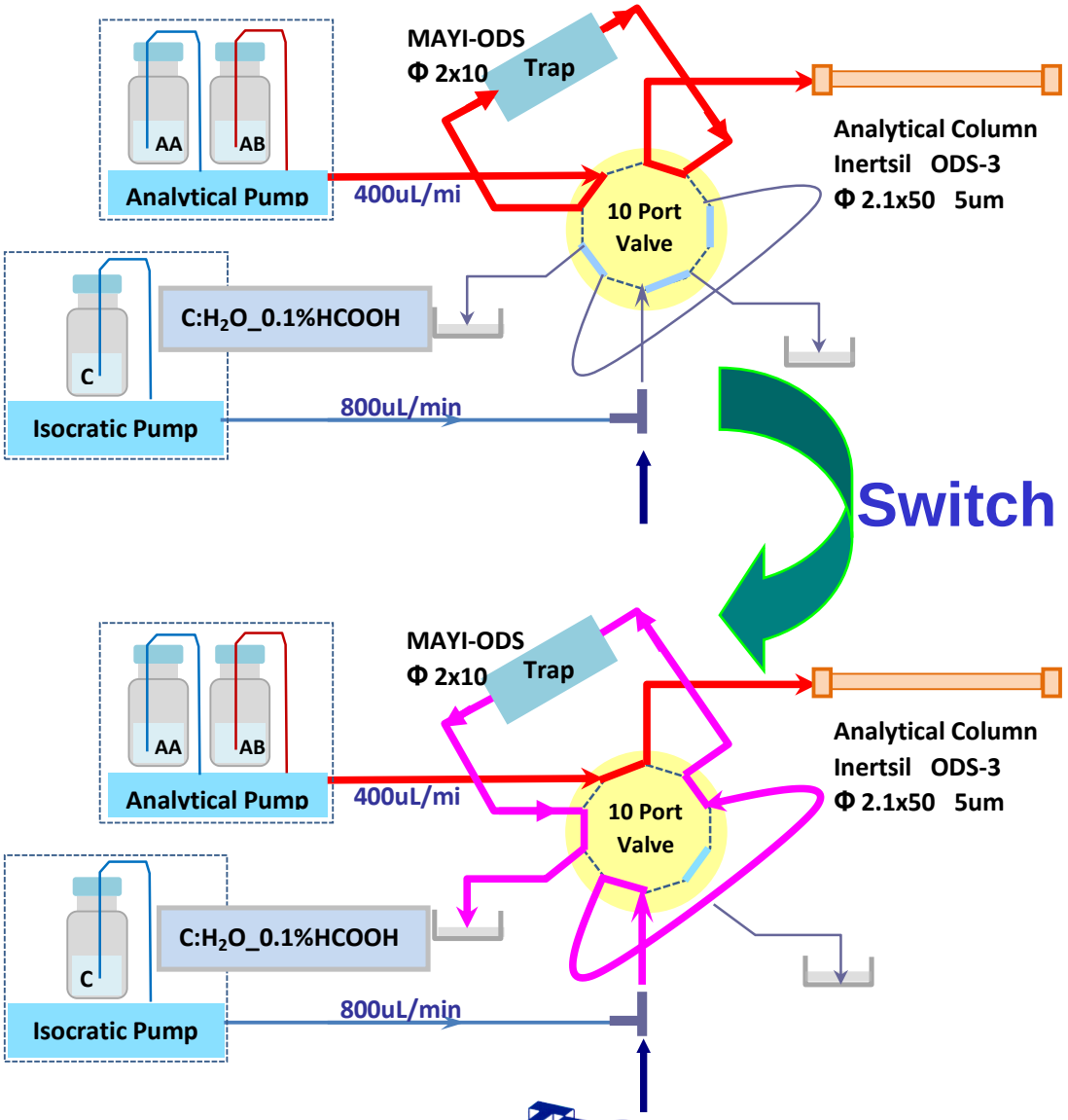
- Improvement reproducibility of placing IS into the sample loop
  e.g.) change to micro syringe from dilutor
- Prevent air bubbles being mixed into dilutor while IS is suctioned
- Apply IS to DBS card in advance
- For reduction of variation in injection volume of IS, larger sample loop volume is preferable
  e.g.) 3 µL → 10 µL

Carry over

Extraction from Trap column

Valve switching

Backwashing



Conclusion

- Information about the effect of DBS cards on Sartan drugs (6 analytes) and statin drugs (2 analytes) was obtained in a short time using HTS method.
- Valsartan in rat whole blood can be quantitated with absolute calibration method.

Future issues

- Improvement the precision of injecting Internal Standard.
- Reduction of peaks derived from carryover.