Optical Resolution through Minimum Derivatization of Chiral Biological Samples

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Background

University

Major : Organic Chemistry

Entered JT

Chemical Research Laboratories

Medicinal Chemist: R&D for small molecule drugs

Toxicology Research Laboratories

Analytical Chemist: R&D for small molecule drugs

Obtained Ph.D : Formation of Bulky DNA Adducts by Non-Enzymatic Production of 1,2-Naphthoquinone-Epoxide from 1,2-Naphthoquinone under Physiological Conditions

Strong area Small molecules and organic chemistry

Topic of JBF

Topics involved in “Bioanalysis”

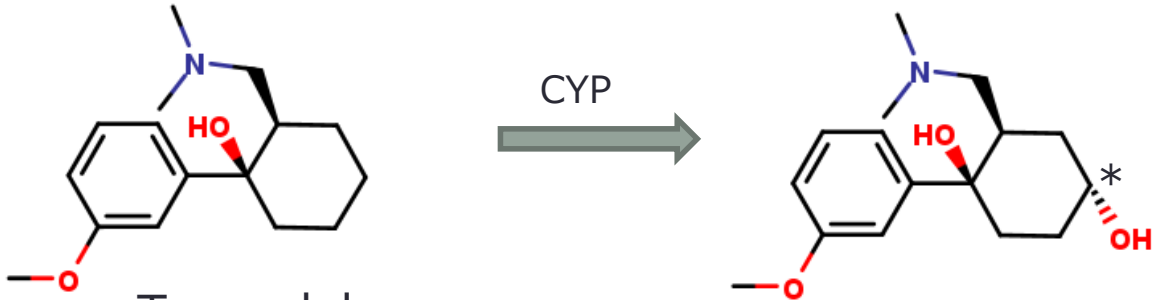
- Bioanalysis
- Guideline
- Method validation
- Biomarker
- LBA analysis

The method I introduce today (developed in our lab.)

- Metabolites of **small molecule drugs**
- Optical resolution
- Derivatization（**Organic chemistry**）

Issues in the analysis of small molecule drugs

Chiral center : generated after metabolization



Tramadol　　Metabolite

Biological samples contain "water".

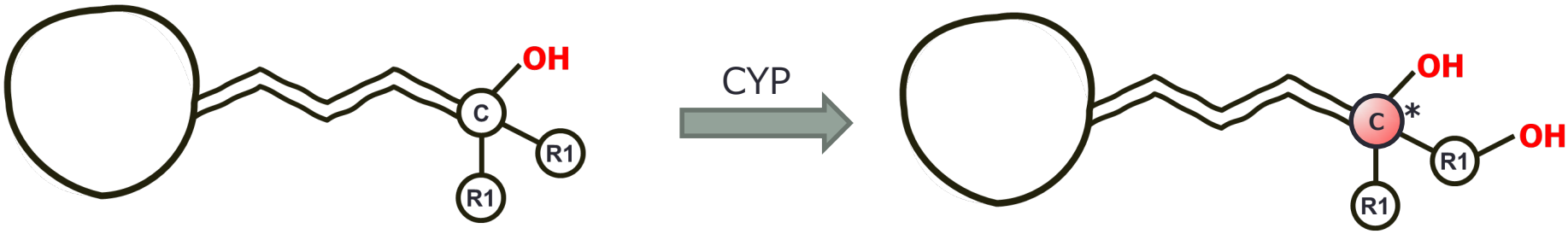
generally・・・

Many samples should be analyzed.

- Chiral column
- Derivatization to diastereomers → separation using conventional columns
  - ✓Derivatization after extraction → needs to be dried
    → liquid-liquid two phase extraction

Many samples should be analyzed.

Our case



Sorry for not showing entire structure・・・。

Enantiomer was produced through CYP oxidation

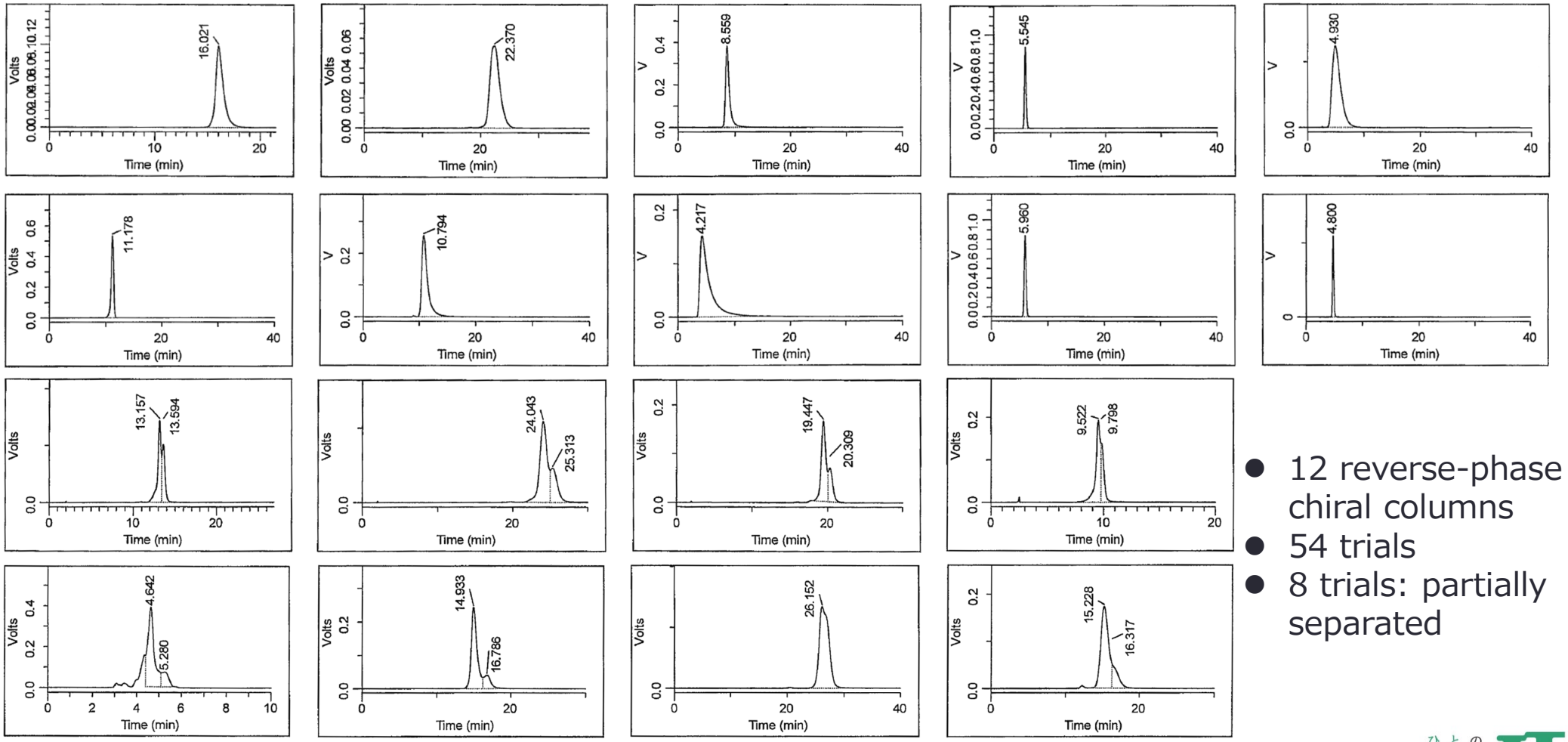
Our impression after watching the structure・・・

- Chiral center was far from the bulky site
- Chiral center was located on alkyl chain with rotatable bonds

Difficult to separate using chiral column

Our trial

Trial using various chiral columns (outsource)



- 12 reverse-phase chiral columns
- 54 trials
- 8 trials: partially separated

Extremely difficult to separate → need to be modified

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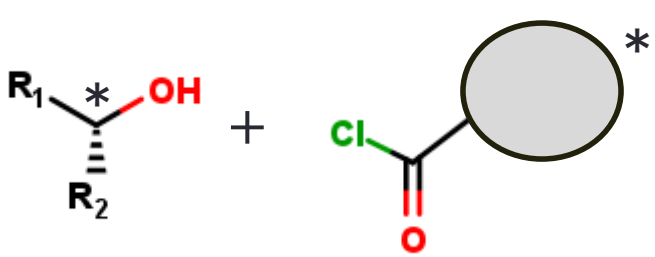
Chiral derivatization

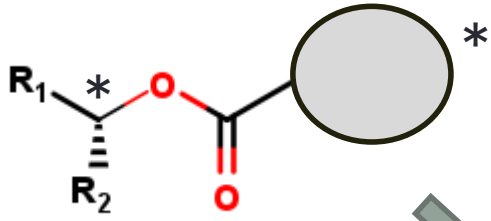
“Common way” is derivatization to diastereomers



Analyte

Chiral acid halides

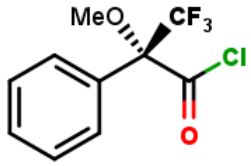
esterification



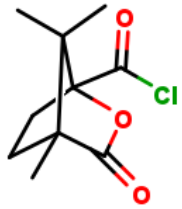
diasteremer

Conventional column

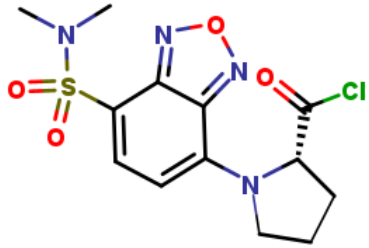
Derivatization reagents



MTPA-Cl
(Mosher reagent)



Camphanic chloride



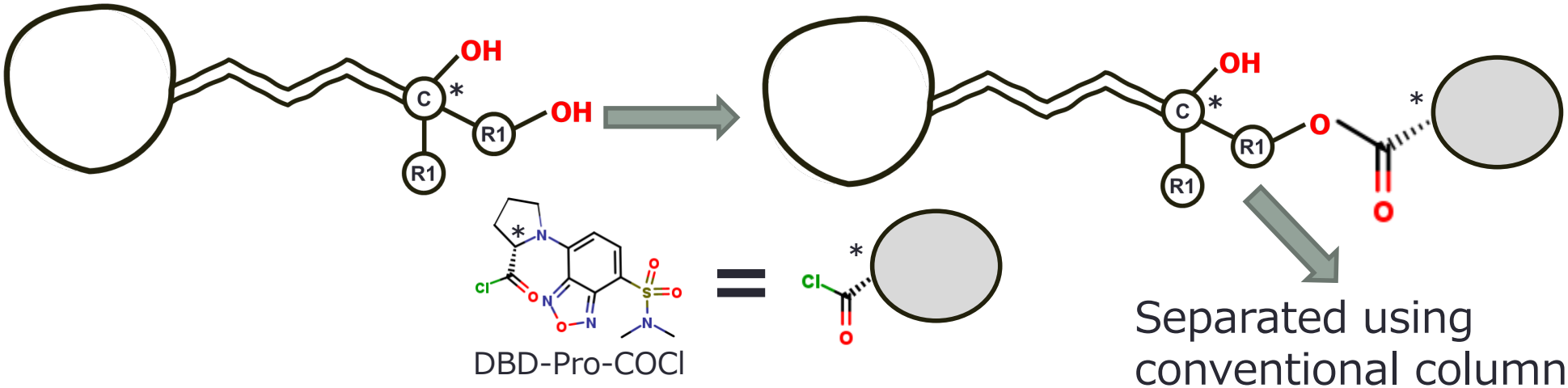
DBD-Pro-COCl

Approach : Chiral derivatization

Derivatization to diastereomer

Esterification using chiral acid chloride

- Not separated completely (about 60%)
- No efficient reaction in the presence of water → need extraction and dry

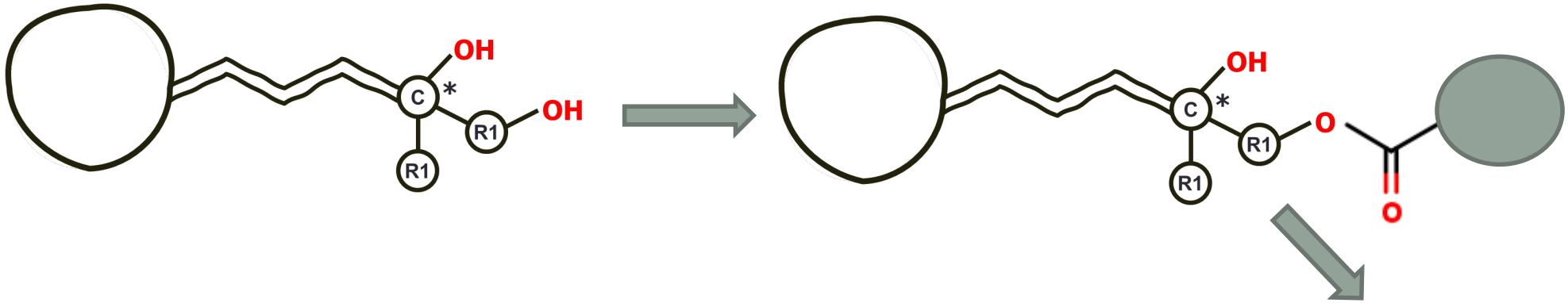


Cons of chiral derivatization

- Acid chlorides: unstable in water, complicated pretreatment
- Reactivity : different reactivities to each enantiomer
- Availability: difficult to obtain in large quantities
- Purity : optical purity of reagent itself

Planning : Non-chiral derivatization

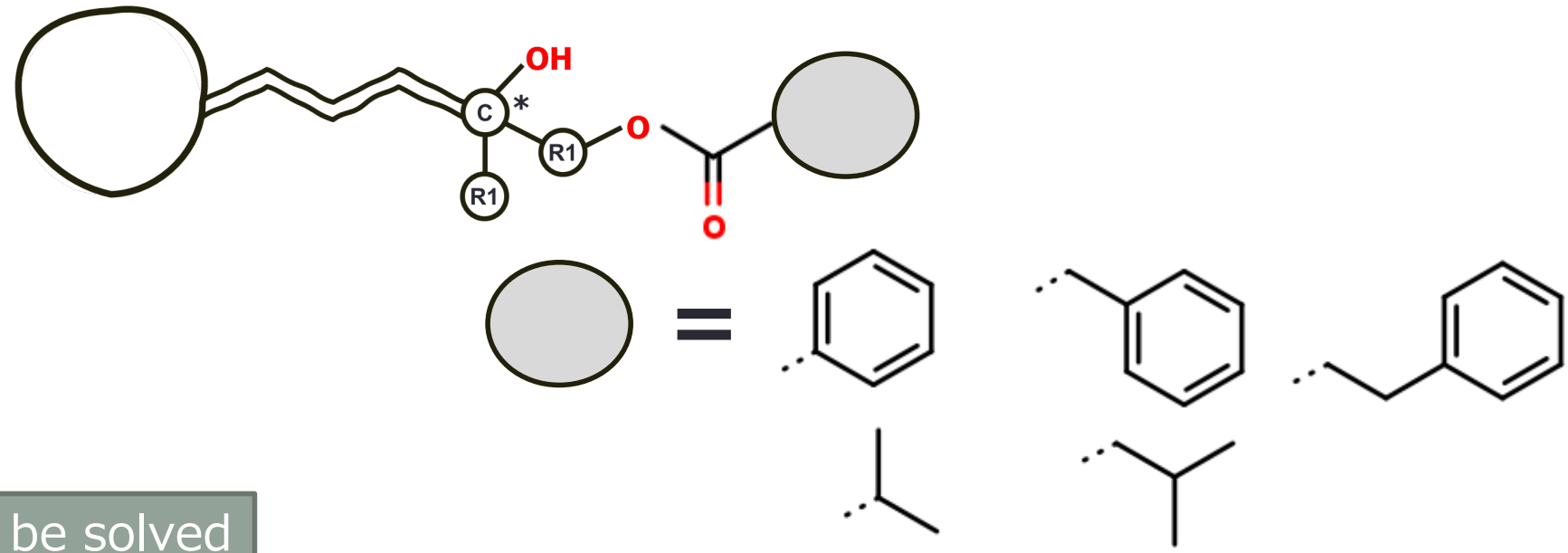
Esterification using non-chiral reagent→ chiral column



Separation using chiral column

- Steric environment → possibly separated by chiral column･･･
- Variety of reagents and conditions → possibly react in the presence of water･･･

Non-chiral derivatization : Trials



Issues to be solved

- Bulkiness ⇔ lipophilicity vs. hydrophilicity
- Water
- Reactivity

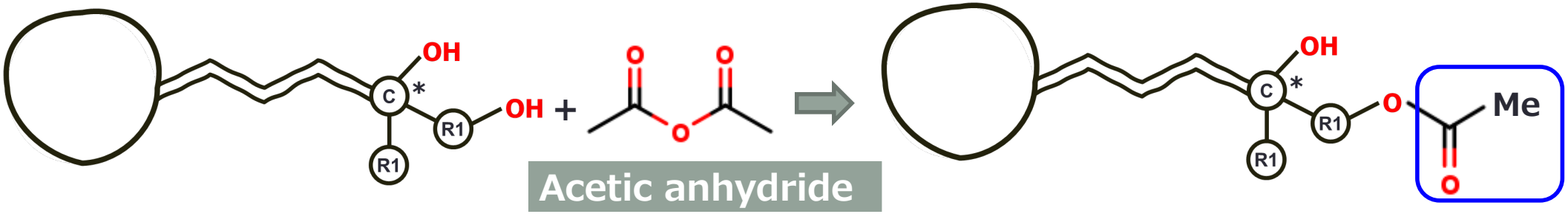
} acid halide, mixed anhydride, anhydride・・・

- Usability : availability, price, irritative, safety

Difficult to meet all the criteria?

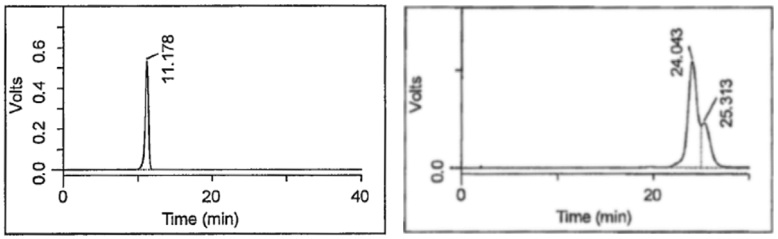
Recognition of **slight** steric environment・・・ ?

Non-chiral derivatization : Acetylization

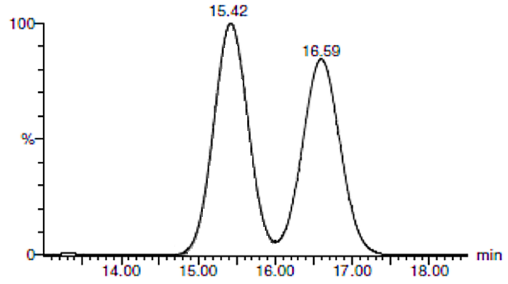


Acetic anhydride

Binding site: estimation



Analyte through chiral column



Chiral column after acetylization

Separation was achieved with acetyl group !

- Separated by minimum derivatization
- Esterified using acetic anhydride

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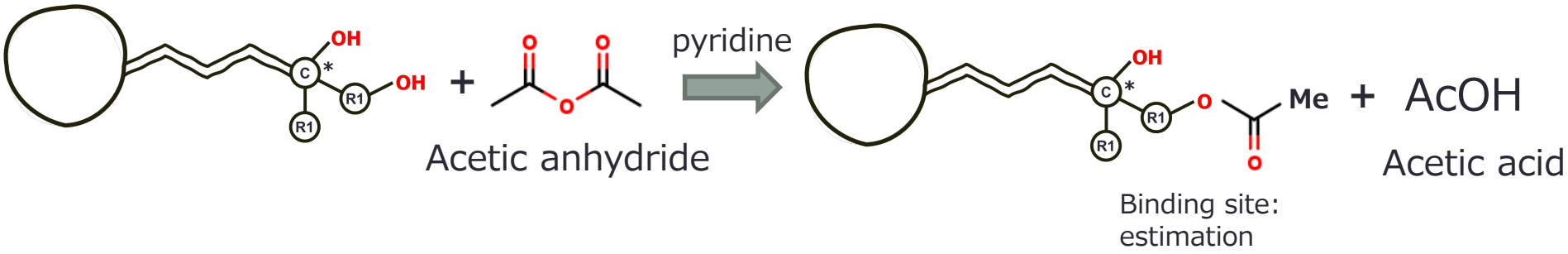
Non-chiral derivatization : Acetylization



Cons of using acetic anhydride

- Compact : High reactivity, reaction time, conversion
- Non-chiral : Similar reactivities to each enantiomer
- Stability in water: Acceptable water containing biological samples
  Easy pretreatment （no evaporation）
- Simple reaction: No need for extra reagents
- By-product (AcOH): Minimal impact on analytical/reaction conditions
- Easily available: Excessive use

Methods

Pretreatment

Analyte 20 µL
- ← MeCN 10 µL
- ← IS 10 µL
- ← MeCN 50 µL

↓ Centrifugation

↓ Supernatant 50 µL
- ← Acetic anhydride 20 µL
- ← Pyridine 30 µL

↓ RT, 1hr

← Water 100 µL

↓ 50℃ 0.5 hr

↓ Centrifugation

↓ Injection 5 µL

LC conditions

Column : CHIRALPAK IC-3, 3 µm, 4.6 ID×150 mm
Column temp. : 40℃
Mobile phase A : Water/FA＝1000/1 (v/v)
Mobile phase B : MeCN/FA＝1000/1 (v/v)

Gradient :

Time (min)	%A	%B	Flow Rate
Initial	70	30	0.6
17.00	70	30	0.6
17.01	5	95	0.6
17.50	5	95	0.6
19.50	5	95	1.0
21.00	5	95	1.0
21.01	70	30	1.0
23.00	70	30	0.6
24.00	70	30	0.6

This method was used for GLP and clinical studies.

Conclusion

Resolution of enantiomer by derivatization with acetic anhydride

- High reactivity
- Similar reactivities to each enantiomer
- Acceptable water containing samples
- By-product is AcOH
- Easily available

This method is extremely useful for quantification of chiral biological samples.

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