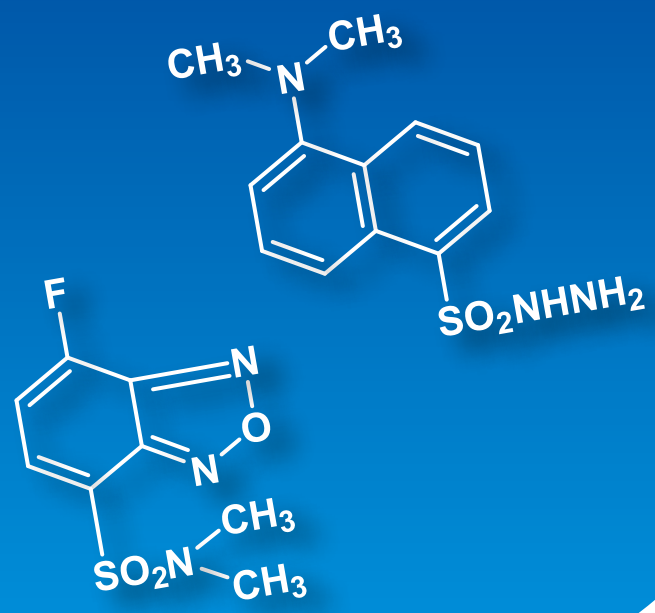
ANALYTICAL

HPLC Labeling Reagents



HPLC Labeling Reagents

HPLC is utilized extensively as a means of detecting and determining trace components. Labeling objective substances for analysis with labeling reagents appropriate for detection methods has been performed in order to obtain higher sensitivity and selectivity. Many labeling reagents have been reported for this purpose. We picked up a part of them and sell them as our TCI-Ace series. All HPLC labeling reagents are high quality products, so you can make use of these products to achieve high quality analyses.

•••••••• Products List by detection and functional groups ••••••••

UV Detection

Fluorescene Detection

for Amino Groups

for Hydroxyl Groups

for Carbonyl Groups

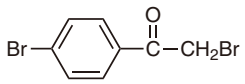
for Thiol Groups

Labeling Reagent for UV Detection

of Carboxylic Acids

4-Bromophenacyl Bromide

5g [A5501]

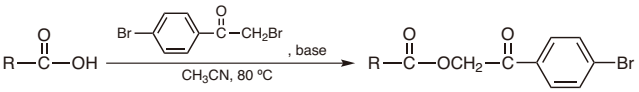


[A5501]

The compound **A5501** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Fatty acids] [1, 2, 8, 9]



Dissolve a sample in methanol or water, and then neutralize the sample solution with methanol solution of KOH-crown ether. Evaporate to dryness under reduced pressure, and then you will see a generally almost white solid substance remaining (potassium salt of fatty acid). Next, add the HPLC labeling reagent **A5501** with acetonitrile solution* of 18-crown 6-ether to this white solid and further add acetonitrile for a volume up to 10 mL. Incubate the solution at 80 °C for 15 min. Cool the resultant solution to room temperature and use it as an HPLC sample.

* Benzene can be used in the place of acetonitrile. The mixing ratio (molar ratio) for the HPLC labeling reagent **A5501** and 18-crown 6-ether should be 20 to 1 and 10 to 1 for the sample fatty acid concentrations at 0.5-20 mM and less than 0.5 mM, respectively. Use the excessive amount of the reagent **A5501**.

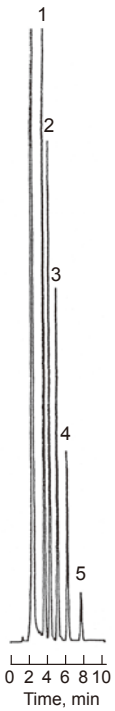
[Others]

Dicarboxyl acids,[2] synthetic prostaglandins,[3] unsaturated fatty acids,[4] alkyl methylphosphonate,[5] ganglioside,6[6] betaine[7]

References

1) H. D. Durst, *Anal. Chem.* **1975**, *47*, 1797.
2) E. Grushka, *J. Chromatogr.* **1975**, *112*, 673.
3) F. A. Fitzpatrick, *Anal. Chem.* **1976**, *48*, 499.
4) Y. Suzuki, T. Takeuchi, Nihon Gakujutsu Shinkoukai Tanka Suiso Kagaku Dai 116 Iinkai Gyoseki Houkoku **1976**, *29*, 152.
5) P. C. Bossle, J. J. Martin, E. W. Sarver, H. Z. Sommer, *J. Chromatogr.* **1983**, *267*, 209.
6) H. Nakabayashi, M. Iwamori, Y. Nagai, *J. Biochem.* **1984**, *96*, 977.
7) S. Konosu, A. Shinagawa, K. Yamaguchi, *Bull. Jpn. Soc. Sci. Fisher.* **1986**, *52*, 869.
8) M. Alberghina, A. Fiumara, L. Pavone, A. M. Giuffrida, *Neurochem. Res.* **1984**, *9*, 1719.
9) K. Kihara, S. Rokushika, H. Hatano, *Bunseki Kagaku* **1984**, *33*, 647.

Chromatogram of fatty acids as 4-bromophenacyl esters



Column : Kaseisorb LC C_1-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 80 / 20
Detection : UV 254 nm
Flow Rate : 1 mL / min

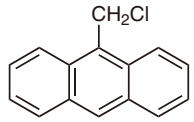
1. Lauric Acid
2. Myristic Acid
3. Palmitic Acid
4. Stearic Acid
5. Arachidic Acid

Labeling Reagent for UV/Fluorescence Detection

of Carboxylic Acids

9-Chloromethylanthracene

1g / 5g [A5502]



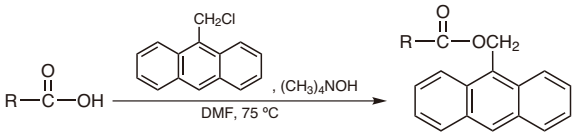
[A5502]

The compound **A5502**, an HPLC labeling reagent which has a chloromethyl group, easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, it has a characteristic fluorescence based on an anthracene skeleton, thus carboxylic acids can be detected with the detection limit of 2 fmol by fluorescence detection analysis at the excitation and emission wavelengths of 365 nm and 412 nm, respectively

Application examples:

[Fatty acids] [1)]

Dissolve 60 µg of a sample in 1 mL of DMF, and add 1 mL of tetramethylammonium hydroxide / DMF solution (1 x 10^{-3} M) and 1 mL of the labeling reagent **A5502** / cyclohexane solution (5 x 10^{-3} M). Close the cap of the reaction vessel and incubate the solution at 75 °C for 30 min. Cool the resultant solution to room temperature and use it as an HPLC sample.



The detection limit = 0.1 pmol (UV detection: 254 nm)

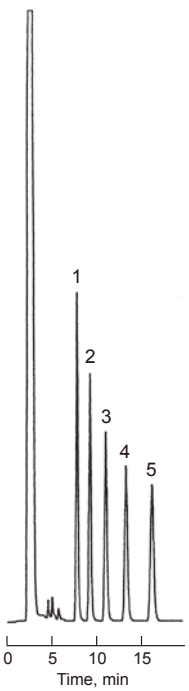
The detection limit = 2 fmol (Fluorescence detection: λex 365 nm, λem 412 nm)

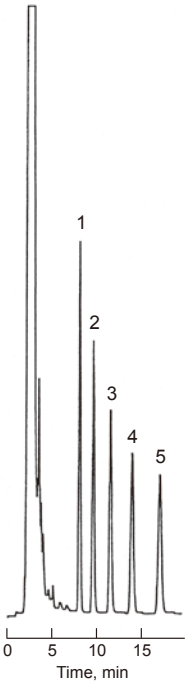
Reference 1) W. D. Korte, *J. Chromatogr.* **1982**, *243*, 153.

Chromatogram of fatty acids as 9-anthrylmethyl esters

Column : Kaseisorb LC C_8-60-5
Column Size
: 4.6 mm I.D.×150 mm
Mobile Phase
: CH_3CN / H_2O = 90 / 10
Detection
: Fluorescence λex 365 nm
λem 412 nm
Flow Rate
: 1 mL / min

1. Lauric Acid
2. *n*-Tridecanoic Acid
3. Myristic Acid
4. *n*-Pentadecanoic Acid
5. Palmitic Acid





Column : Kaseisorb LC C_8-60-5
Column Size
: 4.6 mm I.D.×150 mm
Mobile Phase
: CH_3CN / H_2O = 90 / 10
Detection
: UV 254 nm
Flow Rate
: 1 mL / min

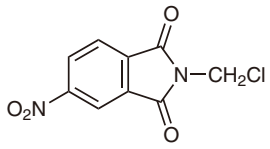
1. Lauric Acid
2. *n*-Tridecanoic Acid
3. Myristic Acid
4. *n*-Pentadecanoic Acid
5. Palmitic Acid

Labeling Reagent for UV Detection

of Carboxylic Acids

N-Chloromethyl-4-nitrophthalimide

1g / 5g **[A5503]**

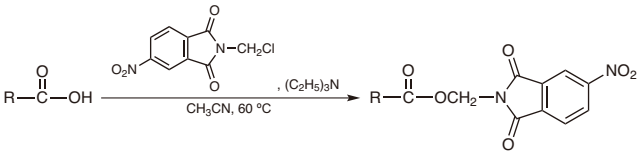


[A5503]

The compound **A5503** is an HPLC labeling reagent, which has a chloromethyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 230 nm.

Application examples:

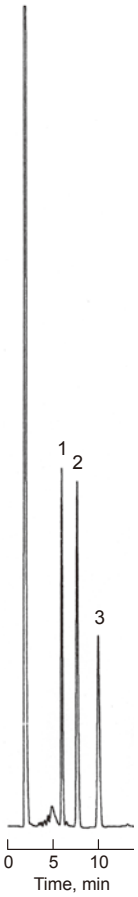
[Fatty acids] [1, 2]



Dissolve 3 mg of a sample in 1 mL of acetonitrile, and add 1 mL of the labeling reagent **A5503** / acetonitrile solution (11 mg/mL) and 1 mL of triethylamine / acetonitrile solution (5 mg/mL). Close the cap of the reaction vessel and incubate the solution at 60 °C for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample. In the case of using alkali metal salts and crown ethers, the esterification reaction is completed in 15 min at 60 °C. Cool the resultant solution to room temperature and use it as an HPLC sample.

References 1) W. Lindner, *J. Chromatogr.* **1979**, *176*, 55.
2) W. Lindner, *J. Chromatogr.* **1980**, *198*, 367.

Chromatogram of fatty acids as (4-nitrophthalimido)methyl esters



Column : Kaseisorb LC ODS-300-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 85 / 15
Detection : UV 230 nm
Flow Rate : 1 mL / min

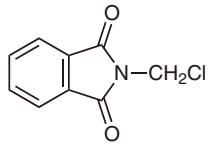
1. Pentadecanoic Acid
2. Palmitic Acid
3. Margaric Acid

Labeling Reagent for UV Detection

of Carboxylic Acids

N-Chloromethylphthalimide

5g [A5504]

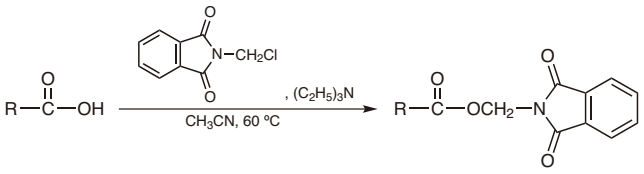


[A5504]

The compound **A5504** is an HPLC labeling reagent, which has a chloromethyl group and easily reacts with a carboxyl group to form an ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

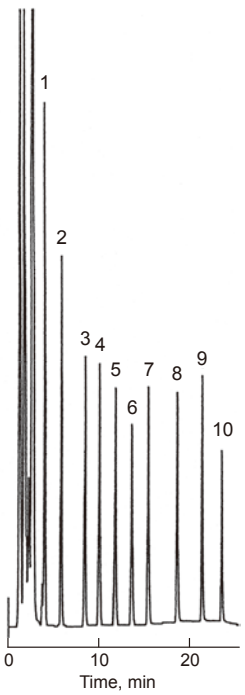
[Fatty acids] [1)]



Dissolve 3 mg of a sample in 1 mL of acetonitrile, and add 1 mL of the labeling reagent **A5504** / acetonitrile solution (10 mg/mL) and 1 mL of triethylamine / acetonitrile solution (5 mg/mL). Close the cap of the reaction vessel and incubate the solution at 60 °C for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample. In the case of using alkali metal salts and crown ethers, the esterification reaction is completed in 5 min at 60 °C. Cool the resultant solution to room temperature and use it as an HPLC sample.

Reference 1) W. Lindner, *J. Chromatogr.* **1979**, *176*, 55.

Chromatogram of fatty acids as phthalimidomethyl esters



Column : Kaseisorb LC C_8-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 70 / 30 → 100 / 0
20 min linear gradient
Detection : UV 254 nm
Flow Rate : 1 mL / min

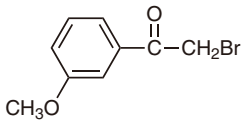
1. *n*-Caproic Acid
2. *n*-Caprylic Acid
3. *n*-Capric Acid
4. *n*-Undecanoic Acid
5. Lauric Acid
6. *n*-Tridecanoic Acid
7. Myristic Acid
8. Palmitic Acid
9. Stearic Acid
10. Arachidic Acid

Labeling Reagent for UV Detection

of Carboxylic Acids

3'-Methoxyphenacyl Bromide

5g [A5505]

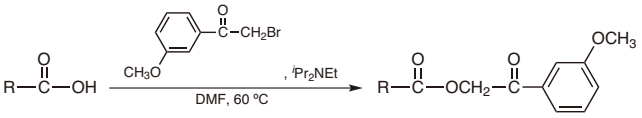


[A5505]

The compound **A5505** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

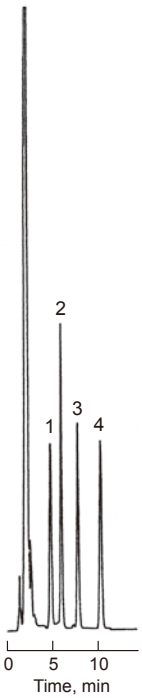
[Fatty acids] [1-3]



Dissolve 4 mg of a sample in 1 mL of *N*,*N*-dimethylformamide (DMF), and add the labeling reagent **A5505** (10 mg) in DMF (1 mL) and *N*,*N*-diisopropylethylamine (10 mg) in DMF (2 mL). Close the cap of the reaction vessel and incubate the solution at 60 ºC for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

References 1) R. A. Miller, N. E. Bussell, C. Ricketts, *J. Liquid Chromatogr.* **1978**, *1*, 291.
2) N. E. Bussell, R. A. Miller, *J. Liquid Chromatogr.* **1979**, *2*, 697.
3) N. E. Bussell, A. Gross, R. A. Miller, *J. Liquid Chromatogr.* **1979**, *2*, 1337.

Chromatogram of fatty acids as 3'-methoxyphenacyl esters



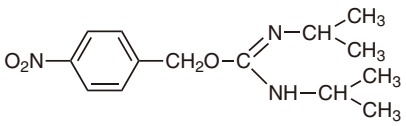
Column : Kaseisorb LC C_8-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detection : UV 254 nm
Flow Rate : 1 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

Labeling Reagent for UV Detection

of Carboxylic Acids

N,N'-Diisopropyl-*O*-(4-nitrobenzyl)isourea 1g [A5506]



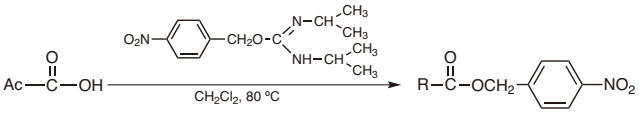
[A5506]

The compound **A5506** easily reacts with a carboxyl group to form the corresponding ester without using a catalyst or an activating agent. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

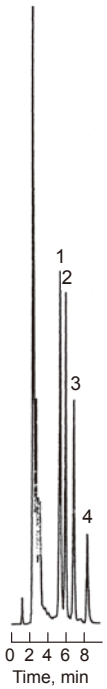
[Fatty acids] [1)]

Dissolve 5 mg of a sample in CH_2Cl_2 (1 mL), and add the labeling reagent **A5506** (20 mg) in CH_2Cl_2 (2 mL). Close the cap of the reaction vessel and incubate the solution at 80 °C for 2 h. Cool the resultant solution to room temperature and use it as an HPLC sample.



References 1) D. R. Knapp, S. Krueger, *Anal. Lett.* **1975**, *8*, 603.
2) B. Sbaikh, N. J. Pontzer, J. E. Molina, M. I. Kelsey, *Anal. Biochem.* **1978**, *85*, 47.
3) S. Okuyama, D. Uemura, Y. Hirata, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 124.
4) R. Badoud, G. Pratz, *J. Chromatogr.* **1986**, *360*, 119.

Chromatogram of fatty acids as 4-nitrobenzyl esters



Column : Kaseisorb LC C_1-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 75 / 25
Detection : UV 254 nm
Flow Rate : 1 mL / min

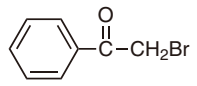
1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

Labeling Reagent for UV Detection

of Carboxylic Acids

Phenacyl Bromide

5g [A5508]



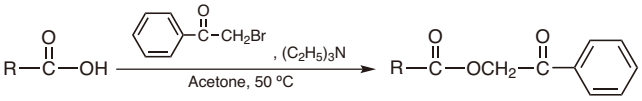
[A5508]

The compound **A5508** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Fatty acids] [1)]

Mix *ca*. 100 µg of a sample, 10 µL of the labeling reagent **A5508** in acetone (12 mg / mL) and 10 µL of triethylamine in acetone (10 mg / mL), and incubate the solution at 50 °C for 2 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

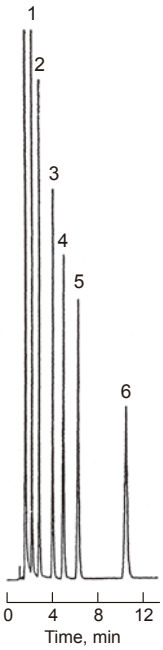


[Others]

Bile acids[2)], fatty acids[3)], carboxylic acids in wine[4)]

References 1) R. F. Borch, *Anal. Chem.* **1975**, *47*, 2437.
2) F. Stellaard, *Anal. Biochem.* **1978**, *87*, 359.
3) K. Kihara, S. Rokushika, H. Hatano, *Bunseki Kagaku* **1984**, *33*, 647.
4) E. Mentasti, M. C. Gennaro, C. Sarzanini, C. Baiocchi, M. Savigliano, *J. Chromatogr.* **1985**, *322*, 177.

Chromatogram of fatty acids as phenacyl esters



Column : Kaseisorb LC ODS-100-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detection : UV 254 nm
Flow Rate : 1 mL / min

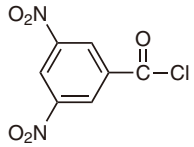
1. Caproic Acid
2. Caprylic Acid
3. Capric Acid
4. Undecanoic Acid
5. Lauric Acid
6. Myristic Acid

Labeling Reagent for UV Detection

of Alcohols and Amines

3,5-Dinitrobenzoyl Chloride

5g [A5511]



[A5511]

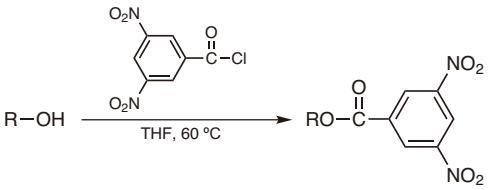
The compound **A5511** is an HPLC labeling reagent, which easily reacts with a hydroxyl group or an amino group to form the corresponding ester or amide, respectively. The resultant ester or amide is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Alcohols] [1)]

Dissolve 1-5 mg of a sample in 5 mL of THF, and add 40 mg of the labeling reagent **A5511** and a few drops of pyridine. Close the cap of the reaction vessel and incubate the solution at 60 °C for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

Clean up before injection is recommended when pyridine or triethylamine is added to trap generated HCl. Generally, evaporate the solvent, extract with ether and wash the ether layer with diluted hydrochloric acid and water.

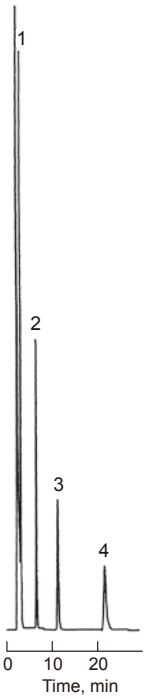


[Others]

Analysis of mono- and diethylene glycols in polyethylene glycol,[2)] aliphatic alcohols[3)]

References 1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.
2) M. A. Carey, H. E. Persinger, *J. Chromatogr. Sci.* **1972**, *10*, 537.
3) Y. Suzuki, N. Tsuchiya, *Bunseki Kagaku* **1981**, *30*, 240.
4) L. J. Elrod, L. B. White, S. G. Spanton, D. G. Stroz, P. J. Cugier, L. A. Luka, *Anal. Chem.* **1984**, *56*, 1786.

Chromatogram of alcohols as 3,5-dinitrobenzoic acid esters



Column : Kaseisorb LC ODS-300-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 55 / 45
Detection : UV 254 nm
Flow Rate : 1 mL / min

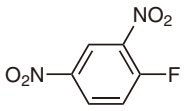
1. Ethylene glycol
2. Ethanol
3. Propanol
4. Butanol

Labeling Reagent for UV Detection

of Amines

2,4-Dinitrofluorobenzene

5mL [A5512]



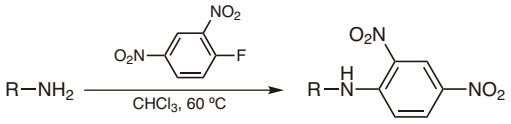
[A5512]

The compound **A5512** easily reacts with an amino group to form the corresponding 2,4-dinitrophenylamine derivative. The resultant derivative is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Amines]

A sample (free amine) 10 mg, chloroform 1 mL, and labeling regent **A5512** (10 eq. excess amount of the sample) are mixed, and incubated at 60 °C for 1 h. After cooling to room temperature, use it as an HPLC sample. **A5512** is also used for derivatization of amino acids.[1,2)]

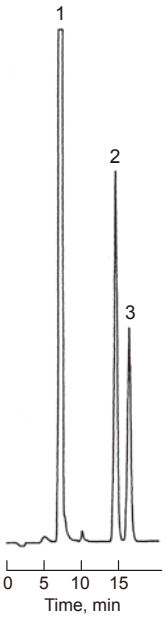


[Others]

Aminoglycosides[3)]

References 1) Y. Suzuki, Program and Abstracts 6th Congress of Liquid Chromatography (October **1985**), 71.
2) S. A. Cockle, H. Kaplan, M. A. Hefford, N. M. Young, 1st High-Perform. Liq. Chromatogr. Proteins Pept., Proc. Int. Symp. **1983**, 103.
3) D. M. Barends, J. S. Blauw, C. W. Mijnsbergen, C. J. L. R. Govers, A. Hulshoff, *J. Chromatogr.* **1985**, *322*, 321.

Chromatogram of alkylamines as 2,4-dinitrophenyl derivatives



Column : Kaseisorb LC C_4-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 45 / 55
Detection : UV 254 nm
Flow Rate : 1 mL / min

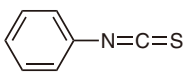
1. Labeling Reagent
2. Diethylamine
3. Propylamine

Labeling Reagent for UV Detection

of Amines

Phenyl Isothiocyanate

5mL [A5513]

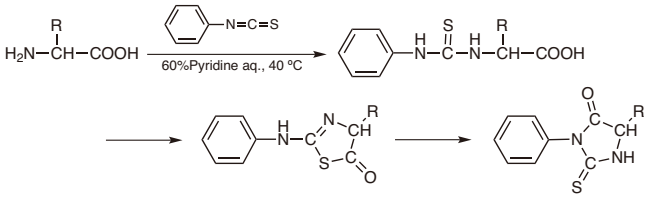


[A5513]

The compound **A5513** is an HPLC labeling reagent, which has an isothiocyano group, can easily react with an amino group to form the corresponding thiourea. The resultant thiourea can be also derivatized into a phenylthiohydantoin (PTH) derivative under acidic conditions. The PTH is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 269 nm for UV detection.

Application examples:

[Amino acids, Peptides]]



1.5 µmol of a sample is dissolved into 1 mL of 60% aqueous pyridine solution containing labeling reagent **A5513** (15 mg), and incubated at 40 °C for 1 h. After cooling to room temperature, the reaction mixture is diluted with 1 mL of water, and excess amount of **A5513** is removed by extraction (benzene 2 mL x 4 times). The aqueous layer is evaporated, and dried in desiccator. To the residue, 1.5 mL of mixed solution (3 N HCl and 60% AcOH, 1 : 1) is added to hydrolyzed at 40 °C for 30 min under a nitrogen atmosphere. After cooling to room temperature, the reaction mixture is diluted with 2 mL of water, and extracted with 2 mL of ethyl acetate, next 2 mL of benzene. The organic layers are combined to use it as an HPLC sample.

References

1) P. Edman, G. Begg, *Eur. J. Biochem.* **1967**, *1*, 80.
2) V. M. Stepanov, *Anal. Biochem.* **1971**, *43*, 209.
3) G. Frank, W. Strubert, *Chromatographia* **1973**, *6*, 522.
4) A. P. Graffeo, *Anal. Lett.* **1973**, *6*, 505.
5) A. Hagg, K. Langern, *Chromatographia* **1974**, *7*, 659.
6) A. P. Graffeo, B. L. Karger, in *Instrumentation in Amino Acid Sequence Analysis*, ed. by R. N. Perham, Academic Press, London, New York, San Francisco, **1975**, p.111.
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8) M. R. Downing, K. G. Mann, *Anal. Biochem.* **1976**, *74*, 298.
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15) M. N. Margolies, A. Brauer, *J. Chromatogr.* **1978**, *148*, 447.
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17) J. Elion, M. Downing, K. Mann, *J. Chromatogr.* **1978**, *155*, 436 .
18) A. S. Bhown, J. E. Mole, W. L. Holloway, C. Bernett, *J. Chromatogr.* **1978**, *156*, 35.
19) R. L. Heinrikson, S. C. Meredith, *Anal. Biochem.* **1984**, *136*, 65.
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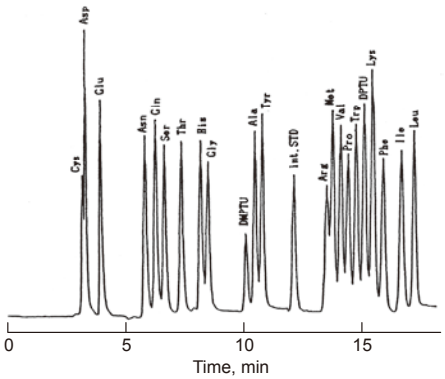
21) R. R. Granberg, *LC, Liq. Chromatogr. HPLC Mag.* **1984**, *2*, 776.
22) B. A. Bidlingmeyer, S. A. Cohen, T. L. Tarvin, *J. Chromatogr.* **1984**, *336*, 93.
23) D. L. Christie, R. M. Hill, K. Isakow, P. M. Barling, *Anal. Biochem.* **1986**, *154*, 92.
24) S. A. Cohen, B. A. Bidlingmeyer, T. L. Tarvin, *Nature (London)* **1986**, *320*, 769.
25) L. E. Lavi, J. S. Holcenberg, D. E. Cole, J. Jolivent, *J. Chromatogr.* **1986**, *377*, 155.
26) D. Lanneluc-Sanson, C. T. Phan, R. L. Granger, *Anal. Biochem.* **1986**, *155*, 322.
27) V. Semensi, M. Sugumaran, *LC-GC* **1986**, *4*, 1108.
28) A. Lilova, T. Kleinschmidt, P. Nedkov, G. Braunitzer, *Biol. Chem. Hoppe-Seyler* **1986**, *367*, 1055.

Chromatogram of amino acids as PTH derivatives



Column	: Kaseisorb LC C_8-60-5
Column Size	: 4.6 mm I.D.×300 mm
Mobile Phase	: A ;CH_3CN
	: B ;40 mM CH_3COONa
	: C ;H_2O
Temperature	: 40 °C
Detection	: UV 269 nm
Flow Rate	: 1 mL / min

Time(min)	A(%)	B(%)	C(%)
0	36	20	44
3	42	20	38
4	45	25	30
5	50	30	20
9	52	30	18
12	65	5	30
13	36	20	44

Labeling Reagent for UV Detection

of Chiral Amines

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Isothiocyanate (= GITC) 100mg / 1g [A5514]

[A5514]

The compound **A5514** is an HPLC labeling reagent for optical purity determination, which has a glyco-moiety and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, **A5514** reacts with a racemic amine to generate diastereomers, which can be efficiently separated by reversed phase HPLC.

Application examples:

[Amino acids] [1)]

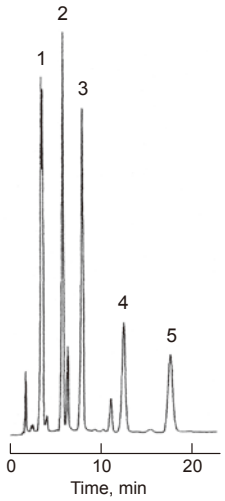
5 mg of an amino acid is dissolved in 50% (V/V) aqueous acetonitrile containing 0.4% (W/V) triethylamine in order to give a final volume of 10 mL. To 50 µL of this solution 50 µL of 0.2% (W/V) labeling reagent **A5514** in acetonitrile are added. The resulting mixture is shaken at room temperature for 30 min and used as an HPLC sample.

[Others]

Propranolol,[2)] trimetoquinol[3)]

References 1) T. Kinoshita, Y. Kasahara, N. Nimura, *J. Chromatogr.* **1981**, *210*, 77.
2) A. J. Sedman, J. Gal, *J. Chromatogr.* **1983**, *278*, 199.
3) H. Nishi, N. Fujimura, H. Yamaguchi, T. Fukuyama, *J. Chromatogr.* **1991**, *539*, 71.

Chromatogram of thiourea derivatives formed from amino acids with GITC



Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : 10 mM Phosphate buffer / Methanol = 45 / 55 (pH 3.0)
Temperature : 25 °C
Detection : UV 254 nm
Flow Rate : 1 mL / min

1. Aspartic Acid
2. L-Valine
3. D-Valine
4. L-Tryptophan
5. D-Tryptophan

Labeling Reagent for UV Detection

of Chiral Amines

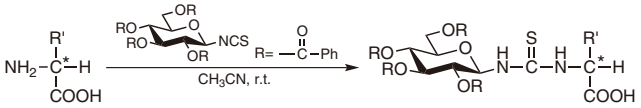
2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl Isothiocyanate 100mg / 1g [A5515] (= BGIT)

[A5515]

The compound **A5515** is an HPLC labeling reagent for optical purity determination, which has a glyco-moiety and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, **A5515** reacts with a racemic amine to generate diastereomers, which can be efficiently separated by reversed phase HPLC.

Application examples:

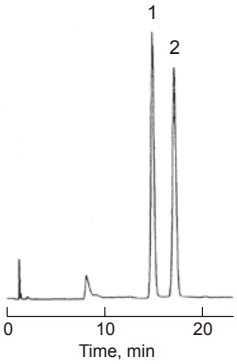
[Amino acids] [1)]



5 mg of an amino acid is dissolved in 50% (V/V) aqueous acetonitrile containing 0.55% (V/V) triethylamine in order to give a final volume of 10 mL. To 50 µL of this solution 50 µL of 0.66% (W/V) labeling reagent **A5515** in acetonitrile are added. The resulting mixture is shaken at room temperature for 30 min, then 10 µL of 0.26% (V/V) ethanolamine in acetonitrile are added and shaken for another 10 min. The mixture is diluted with acetonitrile to a final volume of 1 mL and used as an HPLC sample.

Reference 1) M. Lobell, M. P. Schneider, *J. Chromatogr.* **1993**, *633*, 287.

Chromatogram of thiourea derivatives formed from amino acids with BGIT



Column	: Kaseisorb LC ODS Super
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: 10 mM Phosphate buffer / CH_3CN = 35 / 65 (pH 3.0)
Temperature	: 25 °C
Detection	: UV 254 nm
Flow Rate	: 1 mL / min

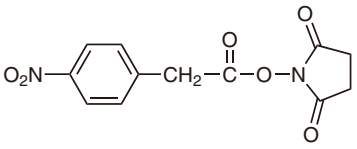
1. L-Phenylalanine
2. D-Phenylalanine

Labeling Reagent for UV Detection

of Amines

N-Succinimidyl 4-Nitrophenylacetate

1g [A5522]

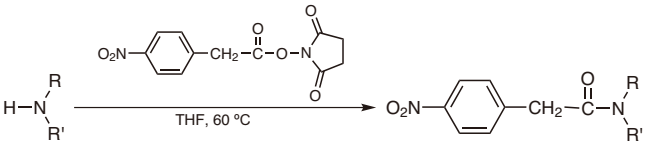


[A5522]

The compound **A5522** is an HPLC labeling reagent, which has a succinimidyl group, which can easily react with an amino group to form the corresponding amide derivative. The resultant amide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Alkylamines]



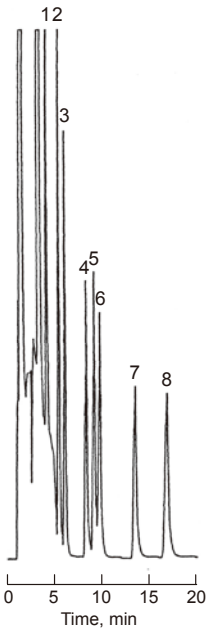
1-5 mg of a sample (free amine), 5 mL of THF, and 50 mg of labeling reagent **A5522** are mixed, and incubated at 60°C for 1 h. After cooling to room temperature, use it as an HPLC sample. If it is necessary to remove the unreacted labeling reagent and by-product, *N*-hydroxysuccinimide, evaporate the solvent at a low temperature under a nitrogen atmosphere. Dissolve the residue in 2-3 mL of ether and wash with aqueous $NaHCO_3$ and water.

[Others]

Drugs (amphetamine, methamphetamine)[1)]

Reference 1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.

Chromatogram of alkylamines as 4-nitrophenylacetamides



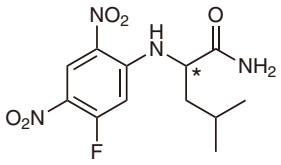
Column : Kaseisorb LC ODS-100-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3OH / H_2O = 60 / 40
Detection : UV 254 nm
Flow Rate : 1 mL / min

1. Propylamine
2. Diethylamine
3. Butylamine
4. Ethylpropylamine
5. Isoamylamine
6. Amylamine
7. Dipropylamine
8. Hexylamine

Labeling Reagent for UV Detection

of Chiral Amines

Nα-(5-Fluoro-2,4-dinitrophenyl)-L-leucinamide (= L-FDLA) 100mg [A5523]
Nα-(5-Fluoro-2,4-dinitrophenyl)-D-leucinamide (= D-FDLA) 100mg / 1g [A5524]



L-form [A5523]
D-form [A5524]

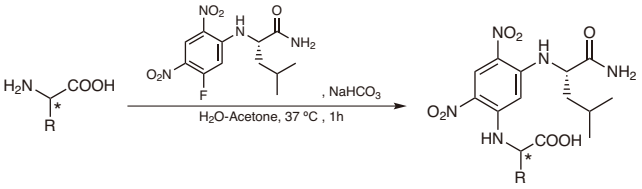
The compounds A5523 and A5524 are HPLC labeling reagents for optical purity determination, and can easily react with amino groups. A5523 or A5524 reacts with a racemic amino acid to generate diastereomers, which can be efficiently separated by reversed phase HPLC. The absolute configuration of amino acids also can be non-empirically determined with use of A5523 and A5524. Furthermore, high sensitive analyses can easily be accomplished using LC-MS. [The detection limit: 5 pmol (ESI LC-MS)]

Application examples:

[Amino acids] [2)]

To 50 µL of a 50 mM aqueous solution of amino acids are added 20 µL of 1 M $NaHCO_3$ and then 100 µL of 1% labeling reagent A5523 or A5524 in acetone. The solution is incubated at 37 °C for 1 h. Reactions are quenched by addition of 20 µL of 1 N HCl. Samples are diluted with 810 µL of acetonitrile, and 1 µL of this solution is analyzed by LC-MS.

Example : L-form



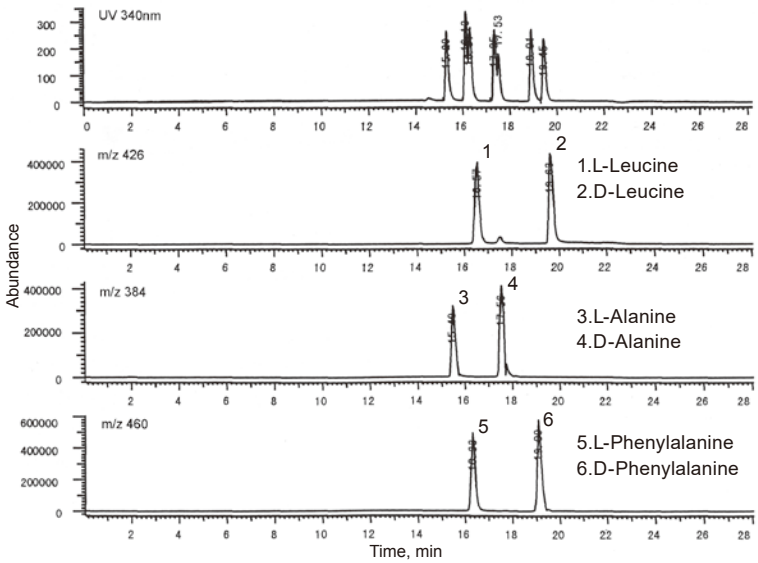
References 1) K. Fujii, Y. Ikai, H. Oka, M. Suzuki, K.-I. Harada, *Anal. Chem.* **1997**, *69*, 5146.
2) K. Fujii, Y. Ikai, T. Mayumi, H. Oka, M. Suzuki, K.-I. Harada, *Anal. Chem.* **1997**, *69*, 346.

Chromatogram of amino acidsas L-FDLA derivatives

Column : Kaseisorb LC ODS 2000
Column Size : 2.0 mm I.D.×150 mm
Mobile Phase : A- 20 mM Ammonium Acetate (pH 4)
B- Methanol

Time(min)	A(%)	B(%)
0	90	10
4	50	50
20	0	100
23	0	100

Temperature : 40 °C
Flow Rate : 0.2 mL / min
Instrument : Hitachi M-8000 LC/3DQ MS
Ionization Method : ESI-AD

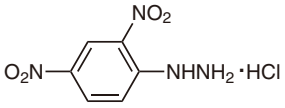


Labeling Reagent for UV Detection

of Carbonyl Compounds

2,4-Dinitrophenylhydrazine Hydrochloride

5g [A5531]



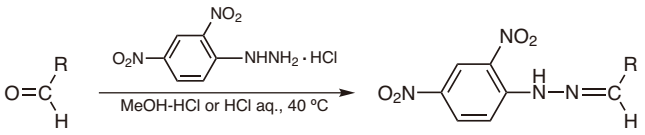
[A5531]

The compound **A5531** is an HPLC labeling reagent, which has a hydrazino group and easily reacts with a carbonyl group to form the corresponding hydrazones. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Aldehydes]

1 mg of a sample, 1 mg of the labeling reagent **A5531**, 1 mL of methanol, and 0.5 mL of 1 N HCl are mixed. Close the cap of the reaction vessel and incubate the mixture at 40 °C for 10 min. After cooling to room temperature, use it as the HPLC sample solution.



[Keto acids] [1,2]

A sample is dissolved in 1 mL of diluted HCl solution containing labeling reagent **A5531** (500 µmol / 2 N HCl 100 mL). Incubate the mixture at 30 °C for 30 min. (The reactions are completed in 5 min and 20 min for ketomonocarboxylic acids and ketodicarboxylic acids, respectively.) It is preferable to add over 4 eq. amount of the labeling reagent, and resultant hydrazones can be extracted with ethyl acetate.

[Urine, 17-Ketosteroids in blood plasma] [3,4]

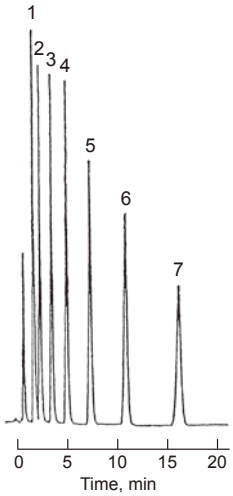
A sample is dissolved into methanol, and acidified with 3-4 drops of conc. HCl. Excess amount of 0.2% labeling reagent **A5531** in methanol is added. Incubate the mixture at 50 °C for 5 min.

[Others]

Aliphatic carbonyl compounds,[5,6] aliphatic aldehydes[7-9]

References
1) H. Katsuki, *Anal. Biochem.* **1968**, *24*, 112.
2) N. Ariga, *Anal. Biochem.* **1972**, *49*, 436.
3) F. A. Fitzpatrick, *Anal. Chem.* **1972**, *44*, 2211.
4) R. A. Henry, *J. Chromatogr. Sci.* **1971**, *9*, 513.
5) M. A. Carey, H. E. Persinger, *J. Chromatogr. Sci.* **1972**, *10*, 537.
6) L. J. Papa, L. P. Turner, *J. Chromatogr. Sci.* **1972**, *10*, 747.
7) Y. Suzuki, H. Maruyama, *Bunseki Kagaku* **1979**, *28*, 671.
8) Y. Suzuki, H. Maruyama, *Bunseki Kagaku* **1985**, *34*, 717.
9) M. Uehori, K. Kuwata, Y. Yamazaki, *Annual report of Environmental Pollution Control Center Osaka Prefecture* **1982**, *5*, 27.

Chromatogram of aldehydes as 2,4-dinitrophenylhydrazones



Column : Kaseisorb LC ODS-60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 70 / 30
Detection : UV 254 nm
Flow Rate : 1 mL / min

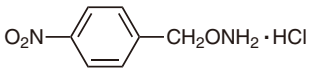
1. Formaline
2. Acetaldehyde
3. Propionaldehyde
4. Butyraldehyde
5. Valeraldehyde
6. Capronaldehyde
7. Heptylaldehyde

Labeling Reagent for UV Detection

of Carbonyl Compounds

O-4-Nitrobenzylhydroxylamine Hydrochloride

1g / 5g **[A5532]**

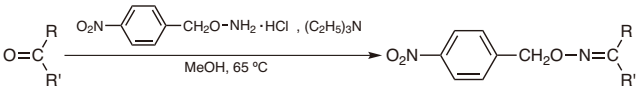


[A5532]

The compound **A5532** is an HPLC labeling reagent, which has a hydroxylamino moiety, can easily react with a carbonyl group to form the corresponding oxime. The resultant oxime is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.

Application examples:

[Aldehydes] [1)]



1-5 mg of a sample, 4 mL of methanol, 2 drops of triethylamine, and 40 mg of the labeling reagent **A5532** are mixed. Close the cap of the reaction vessel and incubate the mixture at 65 °C for 1 h. After cooling to room temperature, use it as the HPLC sample solution. If it is necessary to remove the unreacted labeling reagent and triethylamine, evaporate the solvent at a low temperature under a nitrogen atmosphere. Dissolve the residue in 2-3 mL of ether and wash with diluted HCl and water.

Reference 1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.

Chromatogram of aldehydes as 4-nitrobenzyloximes

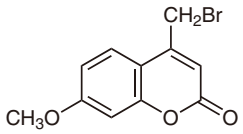
Column : Kaseisorb LC ODS-300-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 60 / 40
Detection : UV 254 nm
Flow Rate : 1 mL / min

1. Propionaldehyde
2. Butyraldehyde
3. Valeraldehyde
4. Capronaldehyde

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

Br-Mmc (= 4-Bromomethyl-7-methoxycoumarin) 1g / 5g [A5551]



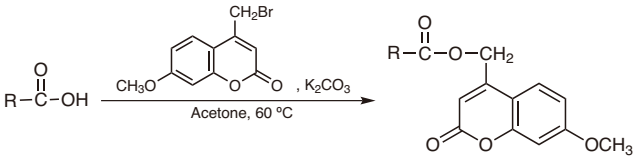
[A5551]

The compound **A5551** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, can easily react with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. Furthermore, it has a characteristic fluorescence based on a coumarin skeleton, thus an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 328 nm and 380 nm, respectively.

Application examples:

[Fatty acids] [1)]

0.05 g of the labeling reagent **A5551** and 0.5 g of K_2CO_3 powder is added to a acetone solution (5 mL) of a sample (0.01 g), and incubate at 60 ºC for 1 h. After cooling to room temperature, use it as the HPLC sample solution.

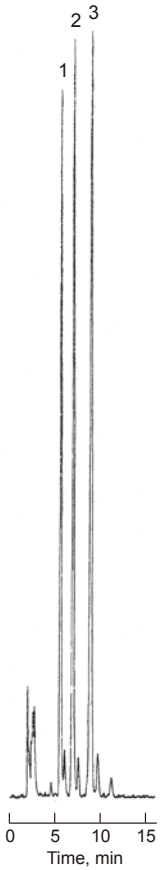


[Others]

Carboxylic acids,[2)] aliphatic acids,[3)] dicarboxylic acids,[4)] prostagrandins,[5)] bile acids,[6)] barbitals[7)]

References 1) W. Dünges, *Anal. Chem.* **1977**, *49*, 442.
2) S. Lam, E. Grushka, *J. Chromatogr.* **1978**, *158*, 207.
3) S. G. Zelenski, J. W. Huber, *Chromatographia* **1978**, *11*, 645.
4) E. Grushka, *Anal. Chem.* **1978**, *50*, 1398.
5) J. Turk, *Prostaglandins* **1978**, *16*, 291.
6) S. Okuyama, *Chem. Lett.* **1979**, 461.
7) W. Dünges, N. Seiler, *J. Chromatogr.* **1978**, *145*, 483.
8) M. L. Grayeski, K. D. Joseph, *Anal. Chem.* **1987**, *59*, 1203.

Chromatogram of fatty acids as methoxycoumarinylmethyl esters



Column : Kaseisorb LC ODS-100-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 85 / 15
Detection : Fluorescence λex 328 nm
λem 380 nm
Flow Rate : 1 mL / min

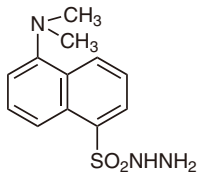
1. Pelargonic Acid
2. Capric Acid
3. Undecanoic Acid

Labeling Reagent for Fluorescence Detection

of Carbonyl Compounds

Dansyl Hydrazine

1g / 5g [A5552]



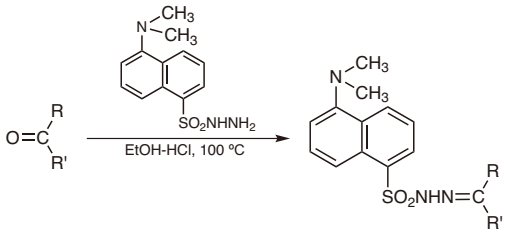
[A5552]

The compound **A5552** is an HPLC fluorescence labeling reagent, and can easily react with a carbonyl group to form the corresponding hydrazone. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 340 nm and 525 nm, respectively.

Application examples:

[Ketosteroids] [1-4]

A dried sample, 0.2 mL of an alcoholic hydrochloric acid (conc. HCl 0.65 mL / ethanol 1 L), and 0.2 mL of the labeling reagent **A5552** in alcohol (2 mg / mL) are mixed, and heated on a water bath for 10 min. 0.2 mL of alcohol containing sodium pyruvate (5 mg / mL) is added to decompose the excess labeling reagent. The reaction mixture is allowed to stand at room temperature for 15 min, ether (6 mL) and 0.5 N NaOH (3 mL) are added and shaken. After an extraction procedure, the solvent is evaporated, chloroform (0.2-0.5 mL) is added to the residue, and use as the HPLC sample.

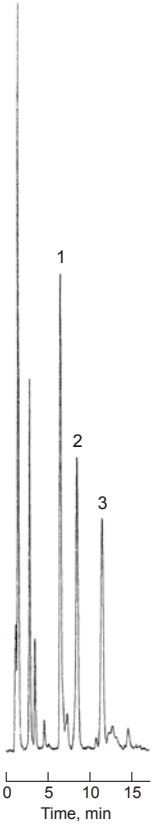


[Others]

Hydrocortisone in body fluid,[3,4] reducing sugars, steroids in serum and urine[5]

References 1) R. Chayen, R. Dvir, S. Gould, A. Harell, *Anal. Biochem.* **1971**, *42*, 283.
2) C. Apter, R. Chayen, S. Gould, A. Harell, *Clin. Chim. Acta* **1972**, *42*, 115.
3) T. Kawasaki, M. Maeda, A. Tsuji, *J. Chromatogr.* **1979**, *163*, 143.
4) T. J. Goehl, G. M. Sundaresan, V. K. Prasad, *J. Pharm. Sci.* **1979**, *68*, 1374.
5) T. Kawasaki, M. Maeda, A. Tsuji, *J. Chromatogr.* **1981**, *226*, 1.

Chromatogram of aldehydes as dansyl hydrazones



Column : Kaseisorb LC ODS-100-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 65 / 35
Detection : Fluorescence λex 340 nm
λem 525 nm
Flow Rate : 1 mL / min

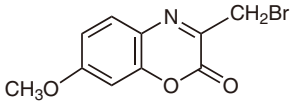
1. Valeraldehyde
2. Capronaldehyde
3. Enanthic Aldehyde

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

3-Bromomethyl-7-methoxy-1,4-benzoxazin-2-one

100mg / 1g **[A5553]**

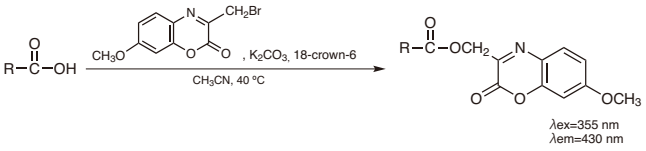


[A5553]

The compound **A5553** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, can easily react with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 355 nm and 430 nm, respectively.

Application examples:

[Fatty acids] [1)]



A solution of the labeling reagent **A5553** (0.1 mL, 1.0 mM acetonitrile solution) is added to a solution of a fatty acid (0.5 mL, 0.2-10 nmol in acetonitrile). To this solution, a saturated K_2CO_3 / acetonitrile solution (0.5 mL) containing 18-crown 6-ether (5.7 mM) is added, and incubate at 40 ºC for 30 min. After cooling to room temperature, use it as the HPLC sample solution.

References 1) H. Naganuma, A. Nakanishi, J. Kondo, K. Watanabe, Y. Kawahara, *Sankyo Kenkyusho Nempo* **1988**, *40*, 51.

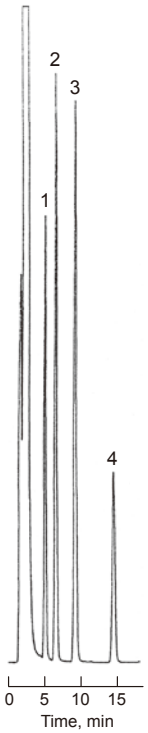
2) A. Nakanishi, H. Naganuma, J. Kondo, K. Watanabe, Y. Kawahara, Program and Abstracts 109th Congress of the Pharmaceutical Society of Japan, 6TA, 2-1.

3) A. Nakanishi, H. Naganuma, J. Kondo, K. Watanabe, K. Hirano, T. Kawasaki, Y. Kawahara, *J. Chromatogr.* **1992**, *591*, 159.

Chromatogram of fatty acids as 7-methoxy-1,4-benzoxazin-2-one-3-methyl esters

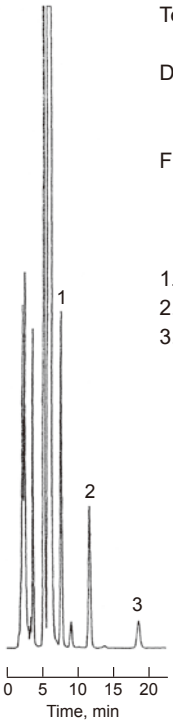
Column : Kaseisorb LC ODS-120-5
Column Size
: 4.6 mm I.D.×150 mm
Mobile Phase
: CH_3CN / H_2O = 95 / 5
Temperature
: 30 ºC
Detection
: Fluorescence λex 355 nm
λem 430 nm
Flow Rate
: 1 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid



Column : Kaseisorb LC ODS-120-5
Column Size
: 4.6 mm I.D.×150 mm
Mobile Phase
: CH_3CN / H_2O = 50 / 50
Temperature
: 25 ºC
Detection
: Fluorescence λex 355 nm
λem 430 nm
Flow Rate
: 1 mL / min

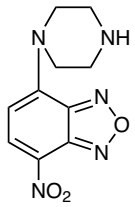
1. Butyric Acid (C_4)
2. Valeric Acid (C_5)
3. Caproic Acid (C_6)



Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

NBD-PZ (= 4-Nitro-7-piperazino-2,1,3-benzoxadiazole) 100mg [A5554]



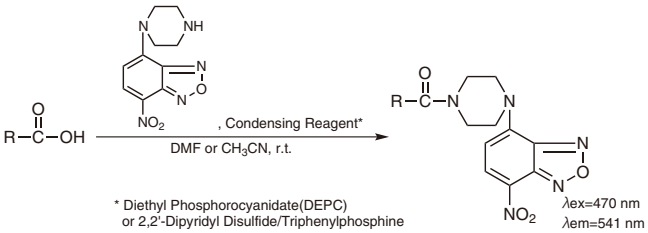
[A5554]

The compound **A5554** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a piperazino group, easily reacts with a carboxyl group at room temperature to form the corresponding amide in the presence of a condensation reagent. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 541 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.

Application examples:

[Fatty acids] [1)]

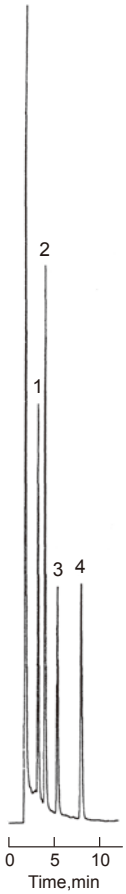
0.2 mL of 140 mM DEPC or 70 mM 2,2'-dipyridyl disulfide / triphenylphosphine / DMF solution containing a fatty acid (10 μM) is added to 0.2 mL of the labeling reagent **A5554** / DMF or acetonitrile solution (10 mM). React at room temperature for 6 h, then use it as an HPLC sample.



* Diethyl Phosphorocyanidate(DEPC)
or 2,2'-Dipyridyl Disulfide/Triphenylphosphine

Reference 1) T. Toyo'oka, M. Ishibashi, Y. Takeda, K. Nakashima, S. Akiyama, S. Uzu, K. Imai, *J. Chromatogr.* **1991**, *588*, 61.

Chromatogram of fatty acids as NBD-PZ derivatives



Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN
Temperature : 25 °C
Detection : Fluorescence λex 470 nm
λem 541 nm
Flow Rate : 1 mL / min

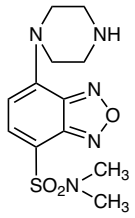
1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

DBD-PZ 100mg [A5555]

[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-piperazino-2,1,3-benzoxadiazole]



[A5555]

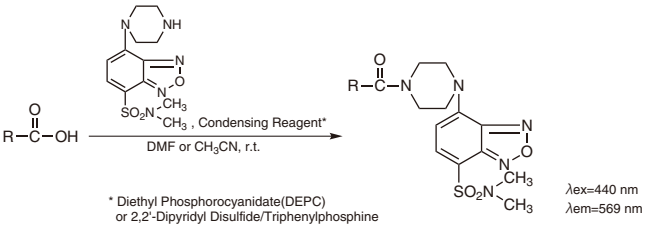
The compound **A5555** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a piperazino group, easily reacts with a carboxyl group at room temperature to form the corresponding amide in the presence of a condensation reagent. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 440 nm and 569 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection[1].

Application examples:

[Fatty acids] [2]

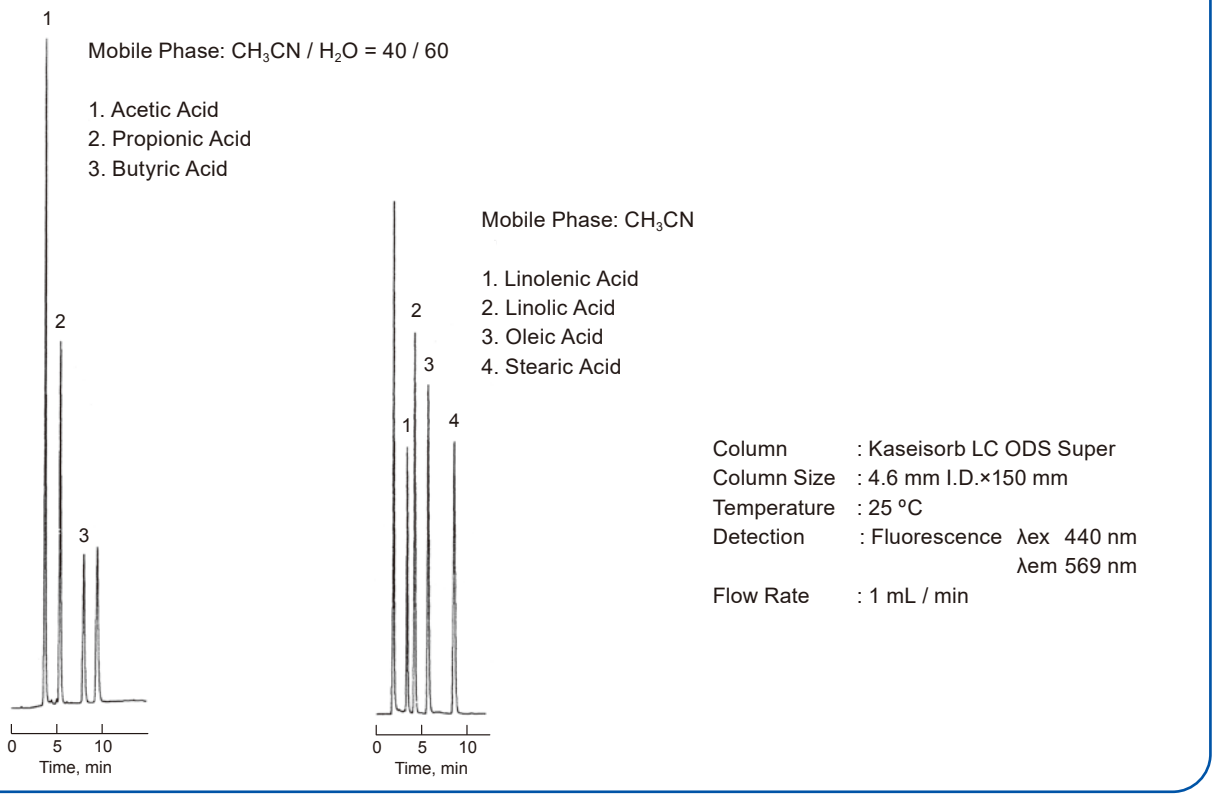
0.2 mL of 140 mM DEPC or 70 mM 2,2'-dipyridyl disulfide / triphenylphosphine / DMF solution containing a fatty acid (10 µM) is added to 0.2 mL of the labeling reagent **A5555** / DMF or acetonitrile solution (10 mM). Incubate at room temperature for 6 h, then use it as an HPLC sample.

For example, the detection limit (S/N = 3) for saturated fatty acids (from C_{13} to C_{24}) is from 3.2 to 4.7 fmol.



References 1) S. Uzu, K. Imai, K. Nakashima, S. Akiyama, *Biomed. Chromatogr.* **1991**, *5*, 184.
2) T. Toyo'oka, M. Ishibashi, Y. Takeda, K. Nakashima, S. Akiyama, S. Uzu, K. Imai, *J. Chromatogr.* **1991**, *588*, 61.

Chromatogram of fatty acids as DBD-PZ derivatives



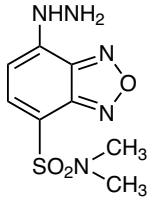
Labeling Reagent for Fluorescence Detection

of Carbonyl Compounds

DBD-H

100mg [A5556]

[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-hydrazino-2,1,3-benzoxadiazole)]



[A5556]

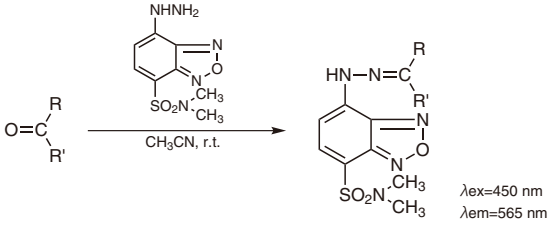
The compound **A5556** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carbonyl group to form the corresponding hydrazone. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 565 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done because of its strong fluorescence.

Application examples:

[Aldehydes or ketones] [1)]

250 µM labeling reagent **A5556** and 1.7 µM propionaldehyde are added to acetonitrile containing 0.025% TFA, and reacted at room temperature for 30 min, then use it as the HPLC sample.

For example, the detection limit for propionaldehyde is 120 fmol.



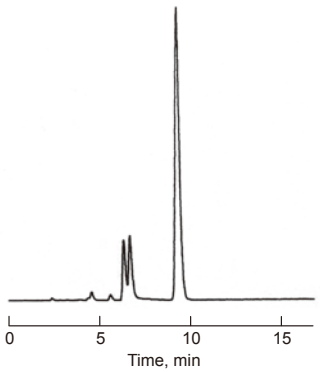
Reference 1) S. Uzu, S. Kanda, K. Imai, K. Nakashima, S. Akiyama, *Analyst* **1990**, *115*, 1477.

Chromatogram of aldehydes and ketones as DBD-H derivatives

Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Temperature : 25 °C
Detection : Fluorescence λex 450 nm
λem 565 nm
Flow Rate : 1 mL / min

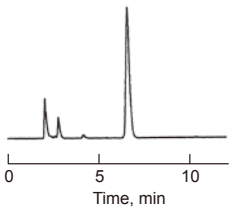
Mobile Phase
: CH_3CN / 0.05% TFA in H_2O = 45 / 55

Propionaldehyde



Mobile Phase
: CH_3CN / 0.05% TFA in H_2O = 70 / 30

Heptan-2-one

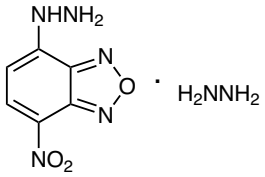


Labeling Reagent for Fluorescence Detection

of Carbonyl Compounds

NBD-H
[= 4-Hydrazino-7-nitro-2,1,3-benzoxadiazole Hydrazine]

100mg [A5557]



[A5557]

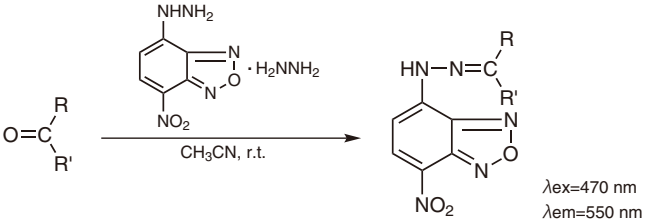
The compound **A5557** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carbonyl group to form the corresponding hydrazone. The labeling reagent itself is non-fluorescent, but the hydrazones after the reaction with carbonyl compounds have strong fluorescence. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 550 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants, and a highly sensitive detection can be done because of its high reactivity.

Application examples:

[Aldehydes or ketones] [1)]

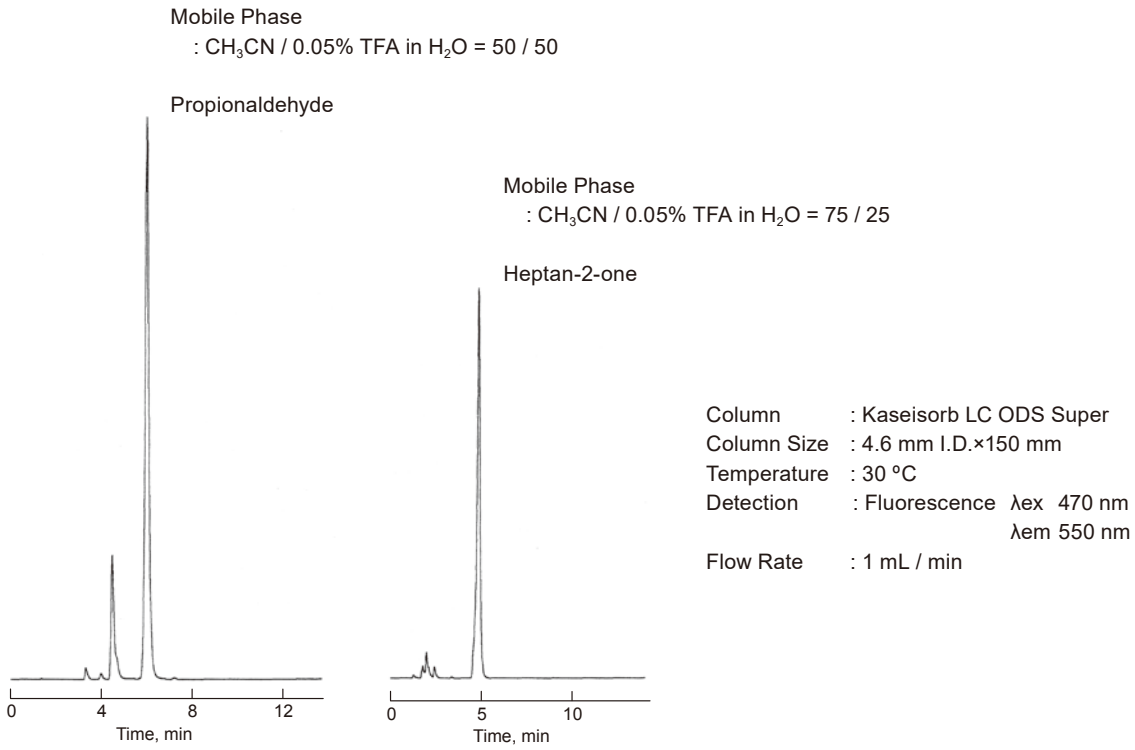
250 µM labeling reagent **A5557** and 1.7 µM propionaldehyde are added to acetonitrile containing 0.025% TFA, and reacted at room temperature for 1 h, use it as the HPLC sample.

For example, the detection limit for propionaldehyde is 35 fmol.



Reference 1) S. Uzu, S. Kanda, K. Imai, K. Nakashima, S. Akiyama, *Analyst* **1990**, *115*, 1477.

Chromatogram of aldehydes and ketones as NBD-H derivatives

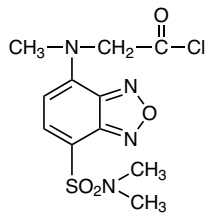


Labeling Reagent for Fluorescence Detection

of Alcohols, Amines and Thiols

DBD-COCl 100mg [A5558]

[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-(*N*-chloroformylmethyl-*N*-methylamino)-2,1,3-benzoxadiazole]



[A5558]

The compound **A5558** is an HPLC fluorescence labeling reagent, which reacts with many kinds of nucleophilic groups under mild conditions. The reaction examples are shown in the table below.

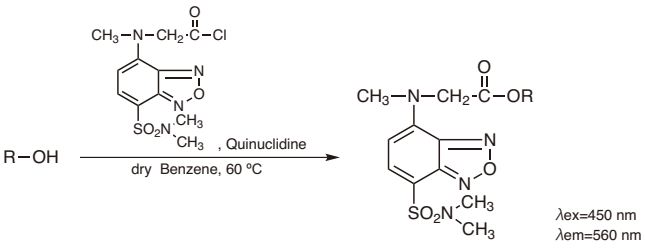
These resulting compounds are stable, and can reach the detector without any decomposition under reversed phase HPLC, thus excellent chromatograms can be obtained by fluorescence detection.

Groups	Examples	Reaction Conditions	Wavelengths (nm)		Detection Limits(fmol)
			ex	em	
Alcohols	Androsterone	60 °C, 30 min	443	546	38
α-Oxyacids	Mandelic acid	60 °C, 15 min	442	551	125
Phenols	Estrone	60 °C, 15 min	440	543	40
Amines	Benzylamine	r.t. or 60 °C, 15 min	445	555	89
Aromatic amines	Phenetidine	60 °C, 15 min	443	553	56
Thiols	2-Mercapto-*N*-(2-naphthyl)-acetamide	r.t.	437	544	103

Application examples:

10 µL of 25 mM labeling reagent **A5558** in dry benzene is mixed with 10 µL of 0.5 mM androsterone in dry benzene (containing 0.5 mM quinuclidine*), and incubated at 60 °C for 30 min. The reaction solution is quenched with 980 µL of 50% acetonitrile solution containing 1% acetic acid, use it as the HPLC sample solution.

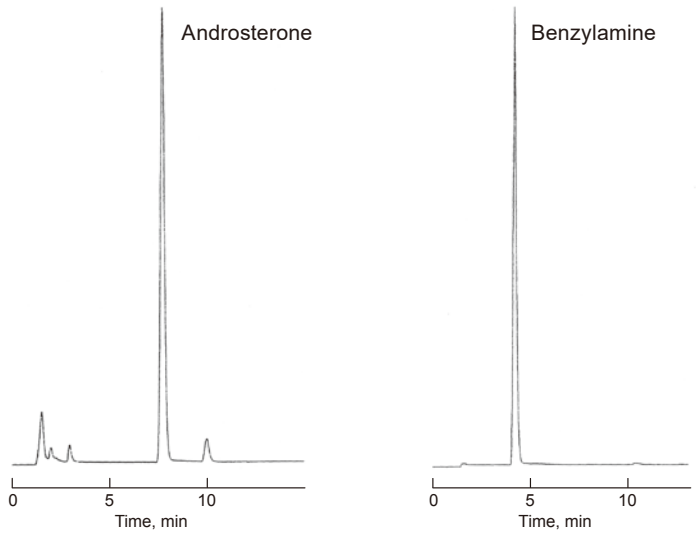
*For primary alcohols, quinuclidine is not necessarily needed.



λex=450 nm
λem=560 nm

References 1) K. Imai, T. Fukushima, H. Yokosu, *Biomed. Chromatogr.* **1994**, *8*, 107.
2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 238075, **1995**.

Chromatogram of alcohol and amine as DBD-COCl derivatives

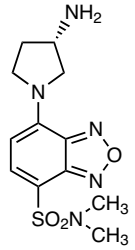


Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 50 / 50
Temperature : 40 °C
Detection : Fluorescence λex 450 nm
λem 560 nm
Flow Rate : 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Carboxylic Acids

(*S*)-(+)-DBD-APy 100mg [A5560]
[= (*S*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



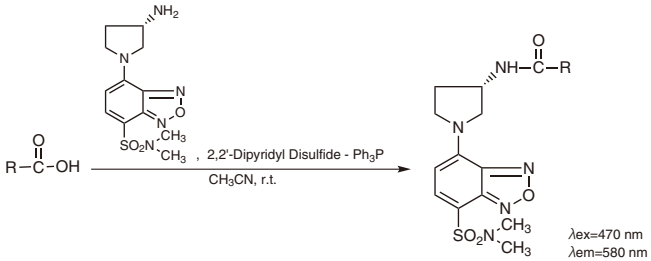
[A5560]

The compound **A5560** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 580 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.

Application examples: [1)]

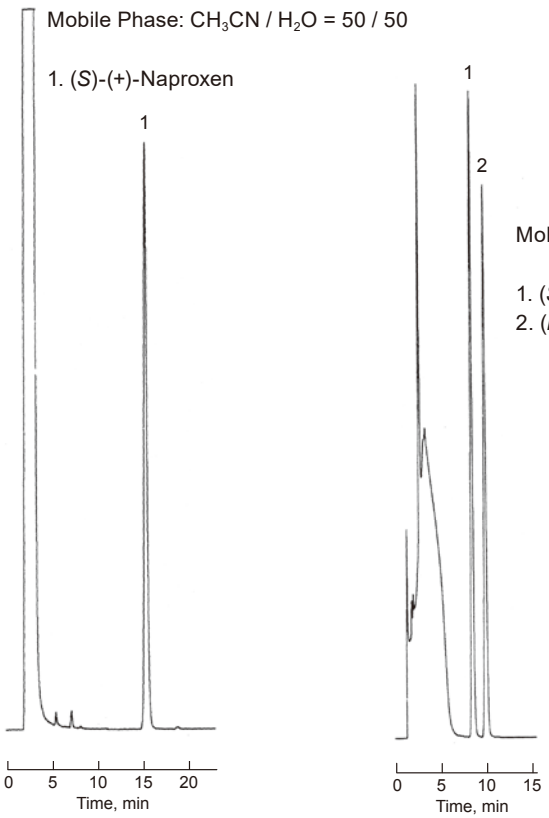
Add 0.1 mL of 10 mM labeling reagent **A5560** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 10 fmol.



References 1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.
2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr. A* **1992**, *625*, 357.

Chromatogram of carboxylic acid enantiomers as (*S*)-(+)-DBD-APy derivatives



Mobile Phase: CH_3CN / H_2O = 60 / 40

1. (*S*)-(+)-Ibuprofen
2. (*R*)-(-)-Ibuprofen

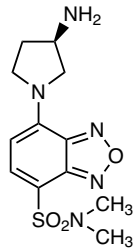
Column	: Kaseisorb LC ODS Super	
Column Size	: 4.6 mm I.D.×150 mm	
Temperature	: 40 °C	
Detection	: Fluorescence	λex 470 nm
		λem 580 nm
Flow Rate	: 1 mL / min	

Labeling Reagent for Fluorescence Detection

of Chiral Carboxylic Acids

(*R*)-(-)-DBD-APy 100mg [A5561]

[= (*R*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



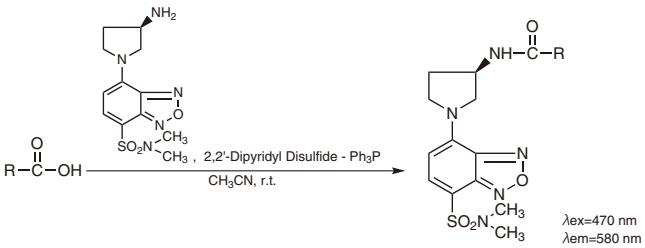
[A5561]

The compound **A5561** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 580 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.

Application examples: [1)]

Add 0.1 mL of 10 mM labeling reagent **A5561** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 10 fmol.

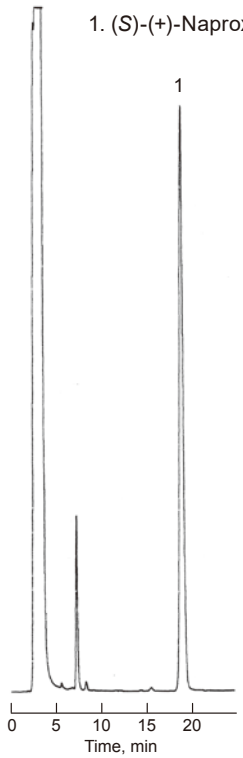


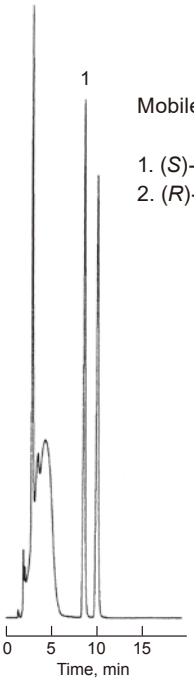
References 1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.
2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr. A* **1992**, *625*, 357.

Chromatogram of carboxylic acid enantiomers as (*R*)-(-)-DBD-APy derivatives

Mobile Phase: CH_3CN / H_2O = 50 / 50

1. (*S*)-(+)-Naproxen





Mobile Phase: CH_3CN / H_2O = 60 / 40

1. (*S*)-(+)-Ibuprofen
2. (*R*)-(-)-Ibuprofen

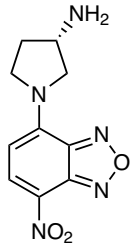
Column	: Kaseisorb LC ODS Super
Column Size	: 4.6 mm I.D.×150 mm
Temperature	: 40 °C
Detection	: Fluorescence λex 470 nm λem 580 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Carboxylic Acids

(*S*)-(+)-NBD-APy 100mg [A5562]

[= (*S*)-(+)-4-Nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



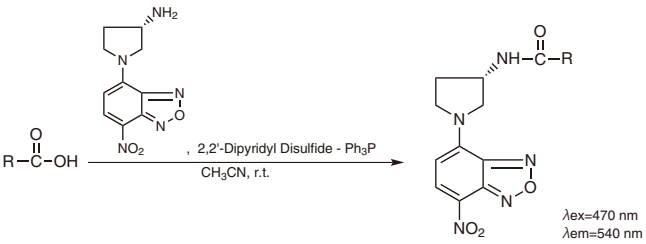
[A5562]

The compound **A5562** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.

Application example: [2)]

Add 0.1 mL of 10 mM labeling reagent **A5562** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

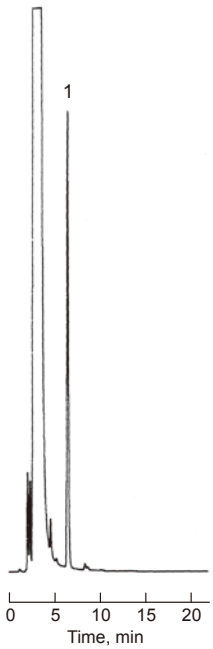
For example, the detection limit (S/N=2) for naproxen is 15 fmol.



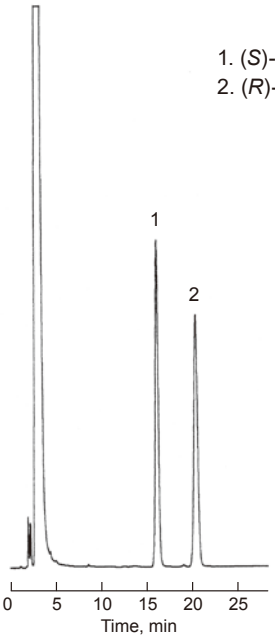
References 1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.
2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr. A* **1992**, *625*, 357.

Chromatogram of carboxylic acid enantiomers as (*S*)-(+)-NBD-APy derivatives

1. (*S*)-(+)-Naproxen



1. (*S*)-(+)-Ibuprofen
2. (*R*)-(-)-Ibuprofen



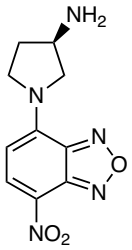
Column	: Kaseisorb LC ODS Super
Column Size	: 4.6 mm I.D.×150 mm
Temperature	: 40 °C
Detection	: Fluorescence λex 470 nm λem 540 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Carboxylic Acids

(*R*)-(-)-NBD-APy 100mg [A5563]

[= (*R*)-(-)-4-Nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



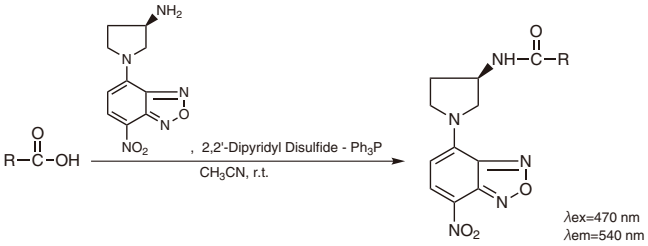
[A5563]

The compound **A5563** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.

Application example: [2)]

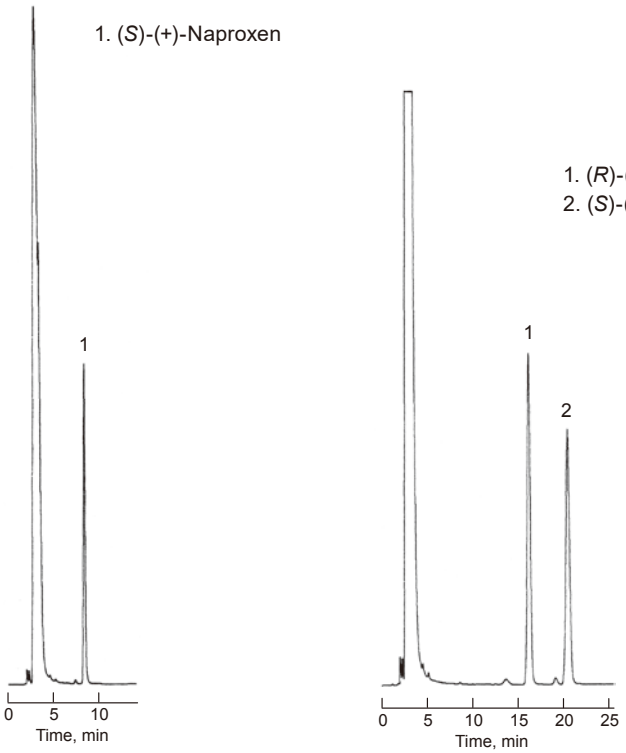
Add 0.1 mL of 10 mM labeling reagent **A5563** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 15 fmol.



References 1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.
2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr.* A **1992**, *625*, 357.

Chromatogram of carboxylic acid enantiomers as (*R*)-(-)-NBD-APy derivatives

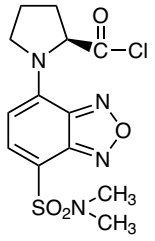


Column	: Kaseisorb LC ODS Super
Column Size	: 4.6 mm I.D.×150 mm
Temperature	: 40 °C
Detection	: Fluorescence λex 470 nm λem 540 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Alcohols and Amines

(*S*)-(-)-DBD-Pro-COCl 100mg [A5564]
[= (*S*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]



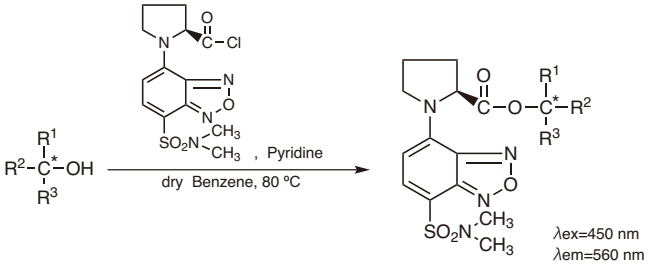
[A5564]

The compound **A5564** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 560 nm, respectively. And the diastereomers derived from racemic alcohol and **A5564** can be separated by HPLC (The separation factor α: 2-hexanol = 1.2). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*R*)-(+)-DBD-Pro-COCl] of **A5564**. The detection limit for the alcohols is sub-picomol.

Application example:

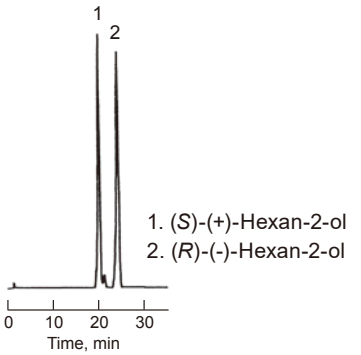
[Secondary alcohols] [1)]

Add 1 mL of 10 mM labeling reagent **A5564** / dry benzene solution and 1 mL of 2 mM alcholol / dry benzene (containing 1% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 °C for 3 h. After cooling to room temperature, excess of **A5564** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.



References 1) T. Toyo'oka, M. Ishibashi, T. Terao, K. Imai, *Analyst* **1993**, *118*, 759.
2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 94 184141, **1994**.

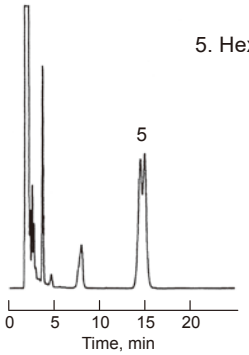
Chromatogram of alcohol enantiomers as (*S*)-(-)-DBD-Pro-COCl derivatives



1. (*S*)-(+)-Hexan-2-ol
2. (*R*)-(-)-Hexan-2-ol

3. (*S*)-(+)- Nonan-2-ol
4. (*R*)-(-)-Nonan-2-ol

Column : Kaseisorb LC 60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : *n*-Hexane / AcOEt = 80 / 20
Temperature : 25 °C
Detection : Fluorescence λex 450 nm
λem 560 nm
Flow Rate : 1 mL / min



5. Hexan-2-ol

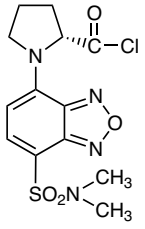
Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 60 / 40
Temperature : 25 °C
Detection : Fluorescence λex 450 nm
λem 560 nm
Flow Rate : 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Alcohols and Amines

(*R*)-(+)-DBD-Pro-COCl [= (*R*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]

100mg [A5565]



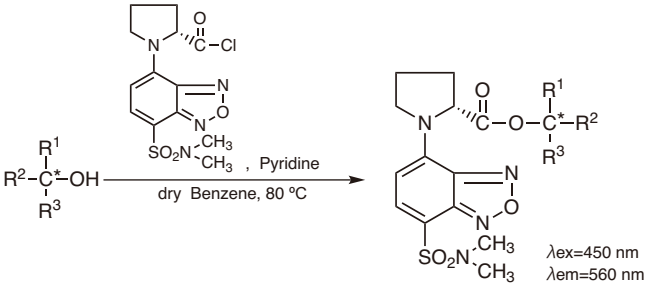
[A5565]

The compound **A5565** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelength of 450 nm and 560 nm, respectively. And the diastereomers derived from racemic alcohol and **A5565** can be separated by HPLC (The separation factor α: 2-hexanol = 1.2). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*S*)-(-)-DBD-Pro-COCl] of **A5565**. The detection limit for the alcohols is sub-picomol.

Application example:

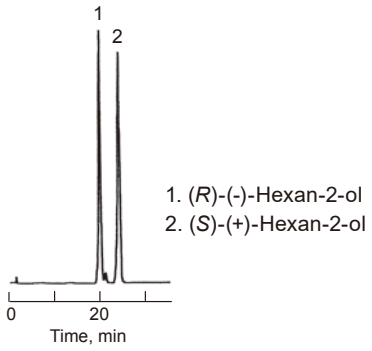
[Secondary alcohols] [1)]

Add 1 mL of 10 mM labeling reagent **A5565** / dry benzene solution, 1 mL of 2 mM alcholol / dry benzene (containing 1% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 °C for 3 h. After cooling to room temperature, excess of **A5565** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.

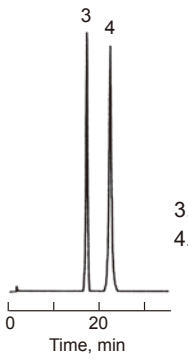


References 1) T. Toyo'oka, M. Ishibashi, T. Terao, K. Imai, *Analyst* **1993**, *118*, 759.
2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 94 184141, **1994**.

Chromatogram of alcohol enantiomers as (*R*)-(+)-DBD-Pro-COCl derivatives

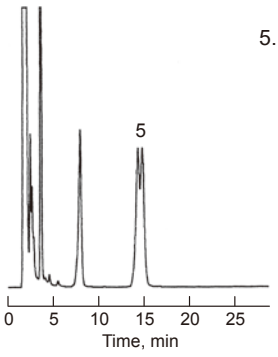


1. (*R*)-(-)-Hexan-2-ol
2. (*S*)-(+)-Hexan-2-ol



3. (*R*)-(-)-Nonan-2-ol
4. (*S*)-(+)- Nonan-2-ol

Column	: Kaseisorb LC 60-5
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: *n*-Hexane / AcOEt = 80 / 20
Temperature	: 25 °C
Detection	: Fluorescence λex 450 nm λem 560 nm
Flow Rate	: 1 mL / min



5. Hexan-2-ol

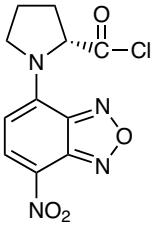
Column	: Kaseisorb LC ODS Super
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 60 / 40
Temperature	: 25 °C
Detection	: Fluorescence λex 450 nm λem 560 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Alcohols and Amines

(*R*)-(+)-NBD-Pro-COCl 100mg [A5566]

[= (*R*)-(+)-4-Nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]

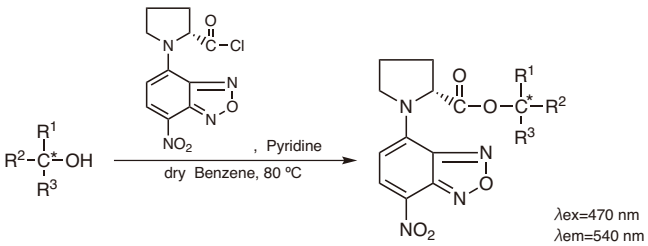


[A5566]

The compound **A5566** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. And the diastereomers derived from racemic alcohol or amine and **A5566** can be separated by HPLC (The separation factor α: 2-hexanol and 1-phenylethylamine = 1.2 and 1.37, respectively). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*S*)-(-)-NBD-Pro-COCl] of **A5566**. The detection limit for the alcohols is sub-picomol. A highly sensitive detection can be done by using laser induced fluorescence detector.

Application example: [1)]

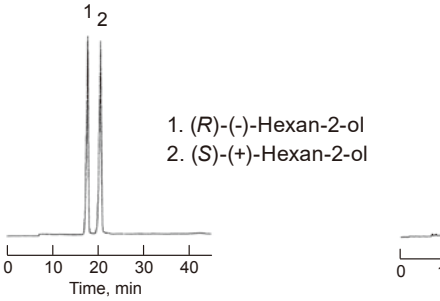
Add 0.5 mL of 40 mM labeling reagent **A5566** / dry benzene solution and 0.5 mL of 1 mM alcholol (or amine) / dry benzene (containing 2% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 °C for 1-2 h (50 °C for 1h, in the case of amine). After cooling to room temperature, excess of **A5566** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.



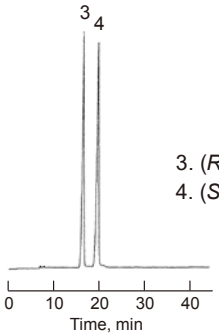
Reference 1) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 188224, **1995**

Chromatogram of alcohol and amine enantiomers as (*R*)-(+)-NBD-Pro-COCl derivatives

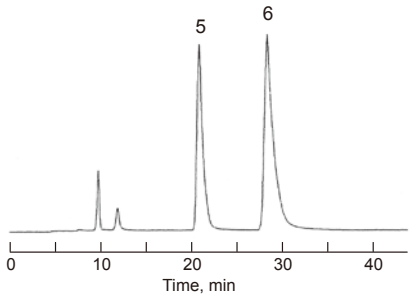
Column : Kaseisorb LC 60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : *n*-Hexane / AcOEt = 80 / 20
Temperature : 40 °C
Detection : Fluorescence λex 470 nm
λem 540 nm
Flow Rate : 1 mL / min



1. (*R*)-(-)-Hexan-2-ol
2. (*S*)-(+)-Hexan-2-ol



3. (*R*)-(-)-Heptan-2-ol
4. (*S*)-(+)-Heptan-2-ol



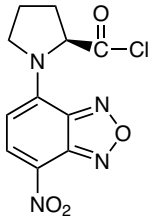
Mobile Phase : *n*-Hexane / AcOEt = 55 / 45

5. (*R*)-(+)-Phenylethylamine
6. (*S*)-(-)-Phenylethylamine

Labeling Reagent for Fluorescence Detection

of Chiral Alcohols and Amines

(*S*)-(-)-NBD-Pro-COCl 100mg [A5567]
[= (*S*)-(-)-4-Nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]

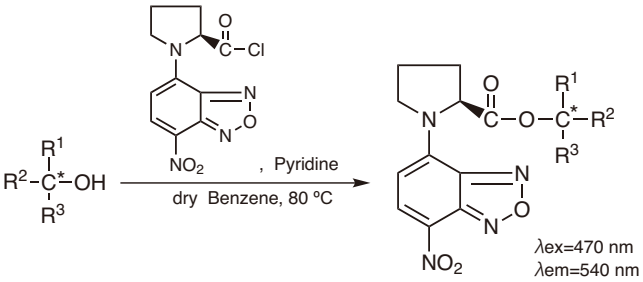


[A5567]

The compound **A5567** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. And the diastereomers derived from racemic alcohol or amine and **A5567** can be separated by HPLC (The separation factor α: 2-hexanol and 1-phenylethylamine = 1.2 and 1.37, respectively). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*R*)-(+)-NBD-Pro-COCl] of **A5567**. The detection limit for the alcohols is sub-picomol. A highly sensitive detection can be done by laser induced fluorescence detector.

Application example: [1)]

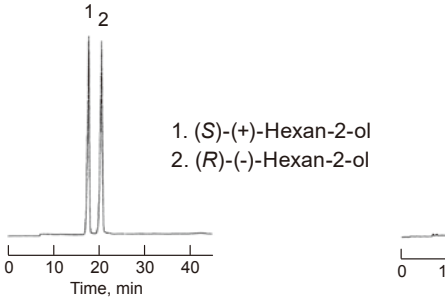
Add 0.5 mL of 40 mM labeling reagent **A5567** / dry benzene solution, 0.5 mL of 1 mM alcholol (or amine) / dry benzene (containing 2% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 °C for 1-2 h (50 °C for 1 h, in the case of amine). After cooling to room temperature, excess of **A5567** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.



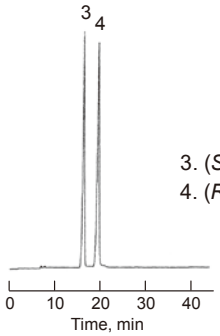
Reference 1) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 188224, **1995**.

Chromatogram of alcohol and amine enantiomers as (*S*)-(-)-NBD-Pro-COCl derivatives

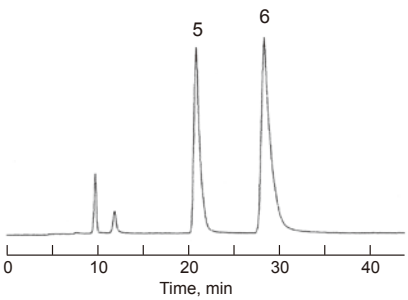
Column : Kaseisorb LC 60-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : *n*-Hexane / AcOEt = 80 / 20
Temperature : 40 °C
Detection : Fluorescence λex 470 nm
λem 540 nm
Flow Rate : 1 mL / min



1. (*S*)-(+)-Hexan-2-ol
2. (*R*)-(-)-Hexan-2-ol



3. (*S*)-(+)-Heptan-2-ol
4. (*R*)-(-)-Heptan-2-ol



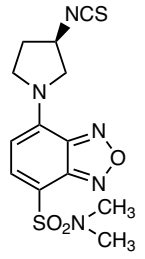
Mobile Phase : *n*-Hexane / AcOEt = 55 / 45

5. (*S*)-(-)-Phenylethylamine
6. (*R*)-(+)-Phenylethylamine

Labeling Reagent for Fluorescence Detection

of Chiral Amines and Thiols

(*R*)-(-)-DBD-Py-NCS 100mg [A5568]
[= (*R*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



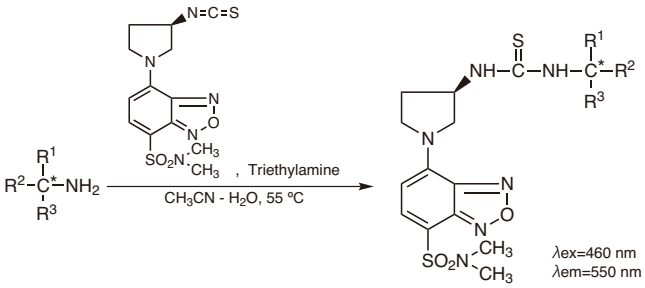
[A5568]

The compound **A5568** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino or mercapto groups, which are directly linked to the asymmetric carbon atom and produces diastereomers of thiourea or dithiocarbamate. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 460 nm and 550 nm, respectively. [The detection limit: thiopronine = 0.5 pmol (S/N = 2)]

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change an elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with a high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.

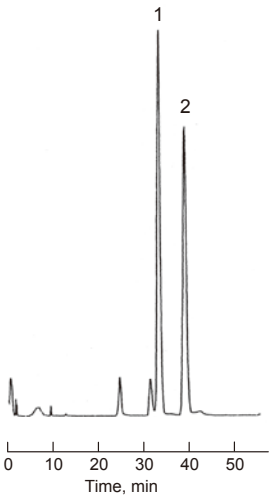
Application example: [1)]

Add 10 µL of 5 mM labeling reagent **A5568** / acetonitrile solution in 10 µL of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel, close the cap of the reaction vessel and incubate the mixture at 55 °C for 10 min. Then, add 480 µL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 µL of this diluted solution as an HPLC sample solution



References 1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.
5) D. Jin, K. Takehana, T. Toyo'oka, *Anal. Sci.* **1997**, *13*, 113.

Chromatogram of amines as (*R*)-(-)-DBD-Py-NCS derivatives



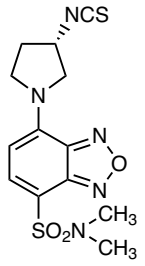
Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60
containing 0.05% TFA
Temperature : Ambient
Detection : Fluorescence λex 460 nm
λem 550 nm
Flow Rate : 1 mL / min

1. (*R*)-1-Phenylethylamine
2. (*S*)-1-Phenylethylamine

Labeling Reagent for Fluorescence Detection

of Chiral Amines and Thiols

(*S*)-(+)-DBD-Py-NCS 100mg [A5569]
[= (*S*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole]



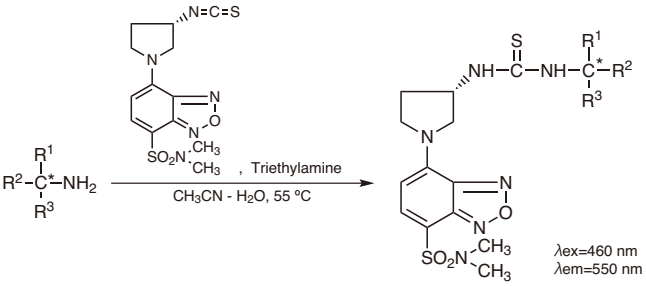
[A5569]

The compound **A5569** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino or mercapto groups, which are directly linked to the asymmetric carbon atom and produces diastereomers of thiourea or dithiocarbamate. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 460 nm and 550 nm, respectively. [The detection limit: thiopronine = 0.5 pmol (S/N = 2)]

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change an elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with a high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.

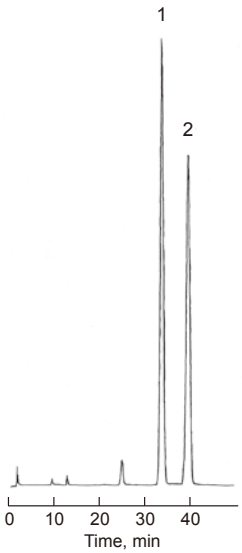
Application example:

Add 10 µL of 5 mM labeling reagent **A5569** / acetonitrile solution in 10 µL of 1 mM amine / acetnitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel, close the cap of the reaction vessel and incubate the mixture at 55 ºC for 10 min. Then, add 480 µL of a mixture solution of 1 M acetic acid and acetnitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 µL of this diluted solution as an HPLC sample solution.



References
1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.
5) D. Jin, K. Takehana, T. Toyo'oka, *Anal. Sci.* **1997**, *13*, 113.

Chromatogram of amines as (*S*)-(+)-DBD-Py-NCS derivatives



Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60
containing 0.05% TFA
Temperature : Ambient
Detection : Fluorescence λex 460 nm
λem 550 nm
Flow Rate : 1 mL / min

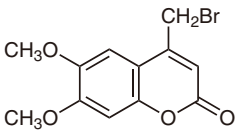
1. (*S*)-1-Phenylethylamine
2. (*R*)-1-Phenylethylamine

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

4-Bromomethyl-6,7-dimethoxycoumarin

100mg / 1g [A5570]

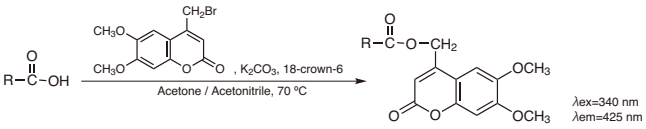


[A5570]

The compound **A5570** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. Furthermore, it has a characteristic fluorescence based on a coumarin skeleton, thus an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 340 nm and 425 nm, respectively.

Application example:

[Fatty acids] [1)]



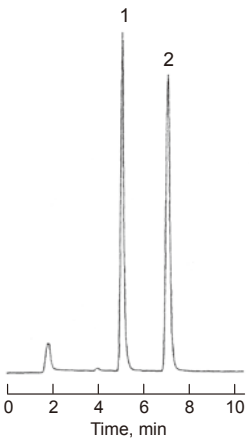
Dissolve 0.01 g of the sample in 0.1 mL of acetone. The solution is neutralized by the addition of 10% KOH / methanol. To the resultant solution, add an acetone solution with an excess amount of labeling reagent **A5570**, 18-crown 6-ether, and potassium carbonate. Close the cap of the reaction vessel and incubate the mixture at 70 °C for 30 min. Cool to room temperature and use it as an HPLC sample solution.

[Others]

Prostaglandins,[1)] bile acids,[1)] proteins,[2)] nucleic acids[3)]

References 1) a) R. Farinotti, Ph. Siard, J. Bourson, S. Kirkiacharian, B. Valeur, G. Mahuzier, *J. Chromatogr.* **1983**, *269*, 81.
b) Y. Amet, F. Berthou, J. F. Menez, *J. Chromatogr. B* **1996**, *681*, 233.
c) A. J. J. M. Coenen, M. J. G. Kerkhoff, R. M. Heringa, Sj. van der Wal, *J. Chromatogr.* **1992**, *593*, 243.
2) a) T. Hiratsuka, *J. Biochem.* **1987**, *101*, 1457.
b) H. I. Stefanova, J. M. East, M. G. Gore, A. G. Lee, *Biochemistry* **1992**, *31*, 6023.
3) a) S. Yoshida, T. Adachi, S. Hirose, *J. Chromatogr.* **1988**, *430*, 156.
b) S. Yoshida, T. Adachi, S. Hirose, *Microchem. J.* **1989**, *39*, 351.

Chromatogram of fatty acids as 6,7-Dimethoxycoumarin 4-methyl esters



Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN
Temperature : Ambient
Detection : Fluorescence λex 340 nm
λem 425 nm
Flow Rate : 1 mL / min

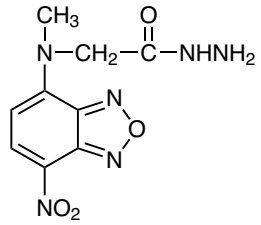
1. Linolic Acid
2. Oleic Acid

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

NBD-CO-Hz
[= 4-(*N*-Hydrazinocarbonylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole]

100mg [A5573]

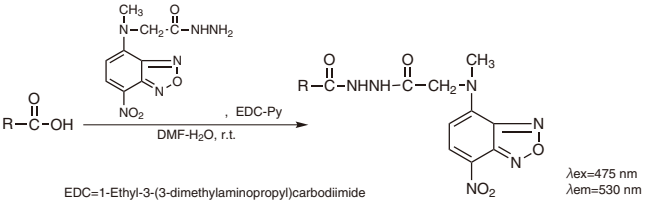


[A5573]

The compound **A5573**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carboxyl group to form the corresponding carbohydrazide in the presence of a condensing agent. The resultant carbohydrazide is stable for at least one week at 4 °C. The carbohydrazide derivatives can be analyzed by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 475 nm and 530 nm, respectively. [The detection limit = 2-4 fmol (S/N = 3)]

Application example:

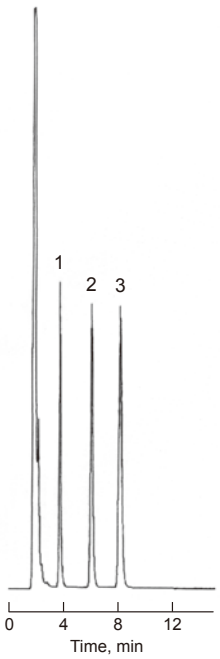
Add 50 µL of carboxylic acid / DMF solution, 50 µL of 1.0 M EDC aqueous solution, 50 µL of 20% pyridine aqueous solution and 20 mM labeling reagent **A5573** / DMF solution to a vessel, and incubate the mixture at room temperature for 2 h. Dilute this reactant mixture 10x with the mobile phase solution, and use 1 µL of this diluted solution as an HPLC sample solution.



EDC=1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

Reference 1) T. Santa, A. Takeda, S. Uchiyama, T. Fukushima, H. Homma, S. Suzuki, H. Yokosu, C. K. Lim, K. Imai, *J. Pharm. Biomed. Anal.* **1998**, *17*, 1065.

Chromatogram of non-steroidal anti-inflammatory drugs as NBD-CO-Hz derivatives



Column	: Kaseisorb LC ODS 2000
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 70 / 30
Temperature	: Ambient
Detection	: Fluorescence λex 475 nm λem 530 nm
Flow Rate	: 1 mL / min

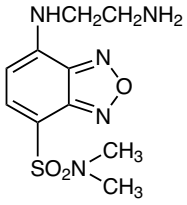
1. Ketoprofen
2. Flurbiprofen
3. Ibuprofen

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

DBD-ED
[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-aminoethylamino)-2,1,3-benzoxadiazole]

100mg [A5574]

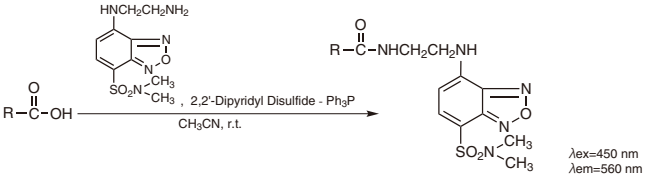


[A5574]

The compound **A5574**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, easily reacts with a carboxyl group to form the corresponding amide in the presence of a condensing agent. The resultant amide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 560 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference from contaminants. Short-chain fatty acids are detectable and determinable reproducibly with a detection limit on the order of fmol. A highly sensitive detection can be done by using chemiluminescence.

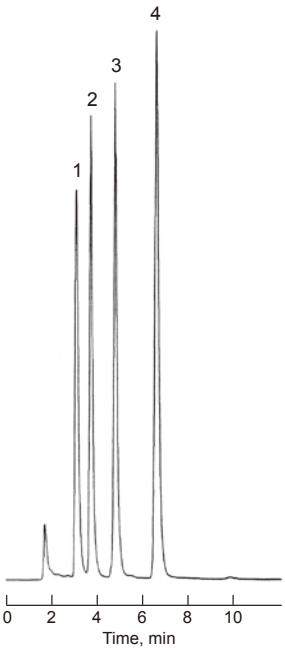
Application example: [2)]

Add 50 µL of mixed fatty acid / diethyl ether solution, 50 µL of 50 mM labeling reagent **A5574** / acetonitrile solution, 50 µL of triphenylphosphine / acetonitrile solution and 50 µL of 2,2'-dipyridyl disulfide / acetonitrile solution to a vessel. This mixture is kept in the dark at room temperature. Dilute this reactant mixture 100x by acetonitrile, and use 10 mL of this diluted solution as an HPLC sample solution.



References 1) Tokyo Kasei Kogyo, Jpn. Kokai Tokkyo Koho 98 218871, **1998**.
2) P. Prados, T. Fukushima, T. Santa, H. Homma, M. Tsunoda, S. Al-Kindy, S. Mori, H. Yokosu, K. Imai, *Anal. Chim. Acta* **1997**, *344*, 227.

Chromatogram of fatty acids as DBD-ED derivatives



Column : Kaseisorb LC ODS 2000
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 5
Temperature : 40 °C
Detection : Fluorescence λex 450 nm
λem 560 nm
Flow Rate : 1 mL / min

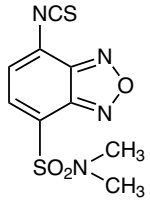
1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

Labeling Reagent for Fluorescence Detection

of Amines

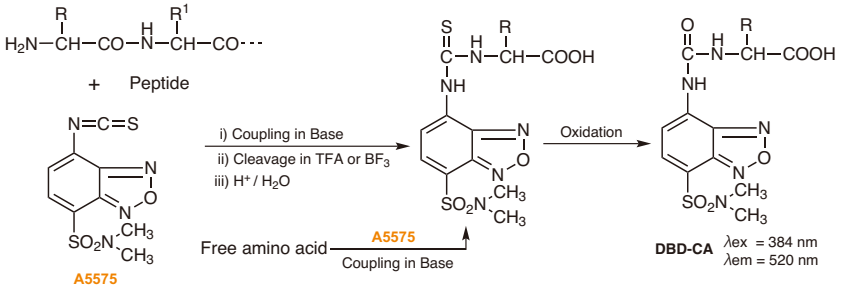
DBD-NCS 100mg [A5575]

[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-isothiocyanato-2,1,3-benzoxadiazole]



[A5575]

The compound **A5575** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 384 nm and 520 nm, respectively. The detection limit for its quantity is an order of sub-picomol (S/N = 3). **A5575** itself does not fluoresce but shows an excellent stability in forms of both crystal and solution, and its derivatives are also stable. This compound can be used for amino acid sequence analysis (Edman Degradation) by binding with the *N*-terminal amino acid of peptides or proteins, followed by acid treatment.



Application example:

[Method by Manual Edman Degradation]

Peptide (insulin Chain B 500 pmol)

- Dissolve in 20 µL of 50% pyridine / H_2O.
- Add 5 µL of 1% triethylamine / CH_3CN and 10 µL of 20 mM HPLC labeling reagent **A5575** / pyridine, and react the mixture at 50 °C for 15 min under the atmosphere of inert gas.
- After cooling to room temperature, wash the reactant solution 3 times with 200 µL of heptane / dichlorormethane (6/4).
- Dry the washed solution at 50 °C for 15 min by using a centrifugation evaporator.
- Add 30 µL of 1% BF_3•Et_2O / CH_3CN to the mixture and incubate the mixture at 50 °C for 5 min.
- Further dry the reactant solution under nitrogen gas.
- Add 20 µL of H_2O, and then extract 2 times with 100 µL of benzene / AcOEt (1/4).

(Aqueous phase)
A peptide will be eluted out.

(Organic phase)

- Dry the extracted organic phase under nitrogen gas
- Dissolve the mixture in 2 µL of CH_3CN.
- Add 8 µL of 0.4 M HCl and hydrolyze the mixture at 50 °C for 5 min.
- Treat the reactant with 5 µL of 4 M HCl and 0.5 M $NaNO_2$ at room temperature for 10 min and oxidize it.
- Neutralize the reactant with 23 µL of 1 M $NaNO_2$, and remove an excessive oxidant by adding 20 µL of 0.15 M methionine.

Use 20 µL of this solution as an HPLC sample solution.

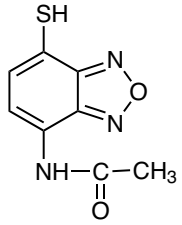
References 1) Y. Huang, H. Matsunaga, A. Toriba, T. Santa, T. Fukushima, K. Imai, *Anal. Biochem.* **1999**, *270*, 257.
2) H. Matsunaga, T. Santa, K. Hagiwara, H. Homma, K. Imai, S. Uzu, K. Nakashima, S. Akiyama, *Anal. Chem.* **1995**, *67*, 4276.
3) K. Imai, S. Uzu, K. Nakashima, S. Akiyama, *Biomed. Chromatogr.* **1993**, *7*, 56.

Labeling Reagent for Fluorescence Detection

of Carboxylic Acids

AABD-SH
[= 4-Acetamido-7-mercapto-2,1,3-benzoxadiazole]

100mg [A5576]

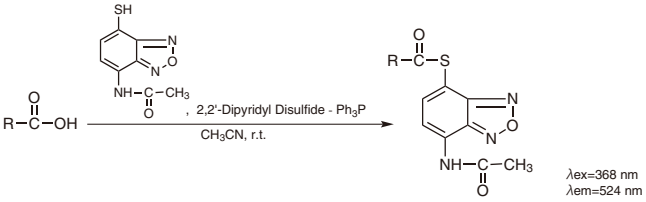


[A5576]

The compound **A5576**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a mercapto group, easily reacts with a carboxyl group to form the corresponding thioester. **A5576** itself fluoresces very little, but the thioester derivatives fluoresce highly. The resultant thioester is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 368 nm and 524 nm, respectively. [The detection limit = 10-20 fmol (S/N = 3)]

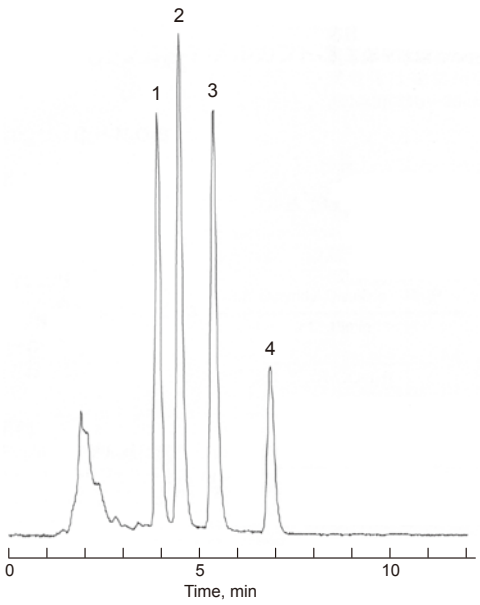
Application example:

Add 20 µL of mixed fatty acid / acetonitrile solution, 20 µL of 20 mM labeling reagent **A5576** / dichloromethane solution, 20 µL of triphenylphosphine / acetonitrile solution and 20 µL of 2,2'-dipyridyl disulfide / acetonitrile solution to a 500 µL vessel, and the mixture is left at room temperature for 15 min. Dilute this reactant mixture with 20 µL of acetonitrile, and use 1 µL of this diluted solution as an HPLC sample solution.



Reference 1) T. Santa, T. Okamoto, S. Uchiyama, K. Mitsuhashi, K. Imai, *Analyst* **1999**, *124*, 1689.

Chromatogram of fatty acids as AABD-thio esters



Column : Kaseisorb LC ODS 2000
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3OH
Temperature : Ambient
Detection : Fluorescence λex 368 nm
λem 524 nm
Flow Rate : 1 mL / min

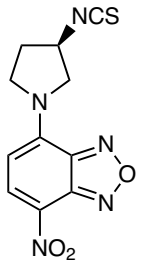
1. Linolenic Acid
2. Linoleic Acid
3. Oleic Acid
4. Stearic Acid

Labeling Reagent for Fluorescence Detection

of Chiral Amines

(*R*)-(-)-NBD-Py-NCS 100mg [A5577]

[= (*R*)-(-)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole]



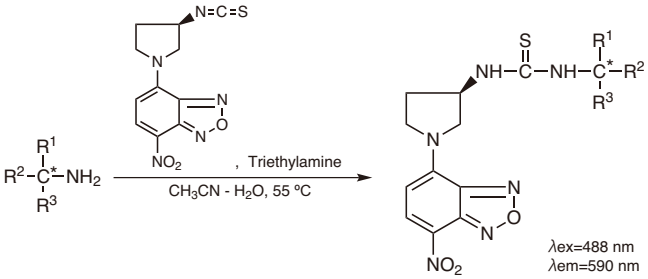
[A5577]

The compound **A5577** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino groups, which are directly linked to an asymmetric carbon atom, and produces diastereomers of thiourea. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 488 nm and 590 nm, respectively.

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change the elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.

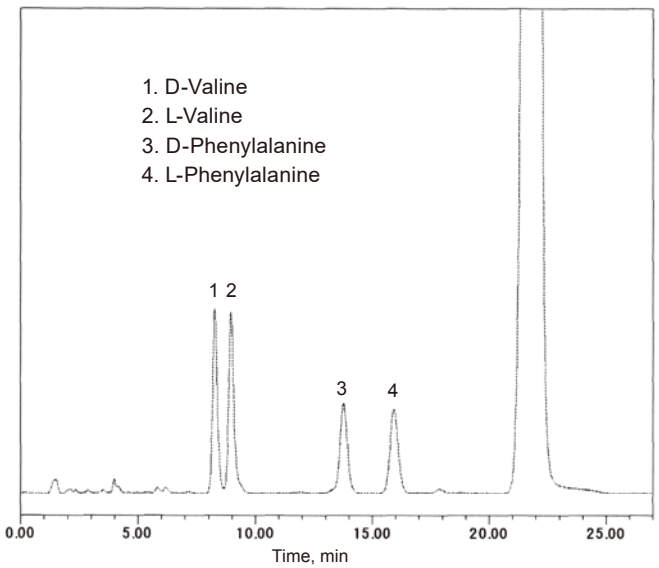
Application example:

Add 10 µL of 5 mM labeling reagent **A5577** / acetonitrile solution in 10 µL of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel. Close the cap of the reaction vessel and incubate the mixture at 55 °C for 10 min. Then, add 480 µL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 µL of this diluted solution as an HPLC sample solution.



References 1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.

Chromatogram of amino acids as (*R*)-(-)-NBD-Py-NCS derivatives

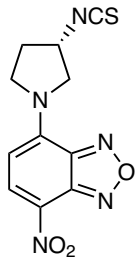


Column	: Kaseisorb LC ODS 2000
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 40 / 60 containing 0.05% TFA
Temperature	: 30 °C
Detection	: Fluorescence λex 488 nm λem 590 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Chiral Amines

(*S*)-(+)-NBD-Py-NCS 100mg [A5578]
[= (*S*)-(+)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole]



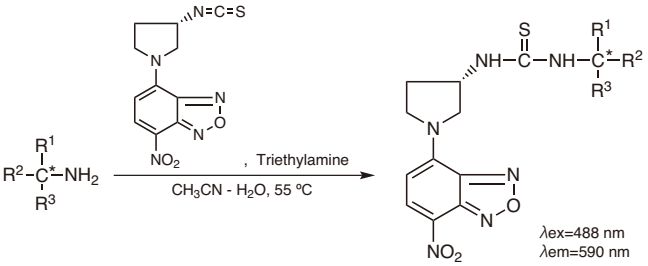
[A5578]

The compound **A5578** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino groups, which are directly linked to an asymmetric carbon atom, and produces diastereomers of thiourea. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 488 nm and 590 nm, respectively.

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change the elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.

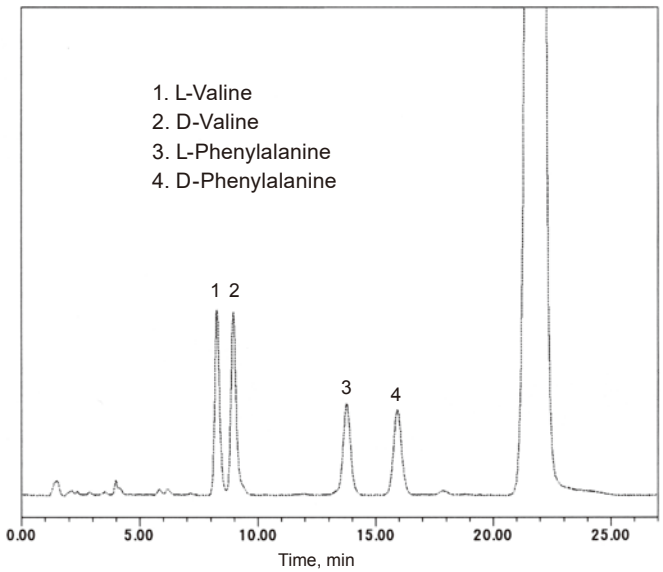
Application example:

Add 10 µL of 5 mM labeling reagent **A5578** / acetonitrile solution in 10 µL solution of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel. Close the cap of the reaction vessel and incubate the mixture at 55 °C for 10 min. Then, add 480 µL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 µL of this diluted solution as an HPLC sample solution.



References
1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.

Chromatogram of amino acids as (*S*)-(+)-NBD-Py-NCS derivatives



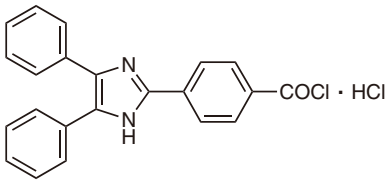
Column	: Kaseisorb LC ODS 2000
Column Size	: 4.6 mm I.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 40 / 60 containing 0.05% TFA
Temperature	: 30 °C
Detection	: Fluorescence λex 488 nm λem 590 nm
Flow Rate	: 1 mL / min

Labeling Reagent for Fluorescence Detection

of Amines and Alcohols

4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzoyl Chloride Hydrochloride

100mg [A5579]



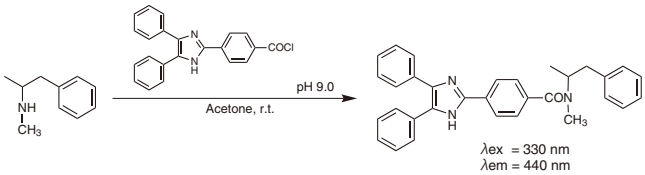
[A5579]

The compound **A5579** is an HPLC fluorescence labeling reagent, which easily reacts with amino groups and hydroxyl groups to form the corresponding amides and esters, respectively. These derivatives are stable for at least 24 h at room temperature, and can reach the detector without any decomposition under reversed phase HPLC. Each derivative can be separated with ODS columns, and the detection limits (S/N = 3) are from 0.6 to 5.2 fmol / 5 µL injection.[1] **A5579** is used for the quantitative analysis of methamphetamine and the derivatives in hair,[3] which is known to preserve drugs for a long term, as well as in urine.[1,2]

Application example:

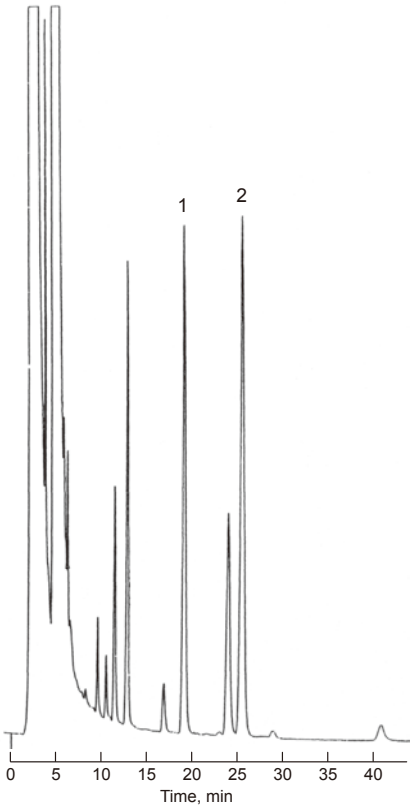
[Quantitative analysis for methamphetamine analogs] [2]

10 µL of urine collected from a methamphetamine addict and 10 µL of acetic acid are put into an amber-glass vial and dried under a flow of nitrogen. 10 µL of carbonate buffer solution and 190 µL of 100 µM labeling reagent **A5579** / acetone solution are added to the residue, reacted at room temperature for 10 min. Use it as an HPLC sample solution.



References 1) O. Al-Dirbashi, J. Qvarnstrom, K. Irgum, K. Nakashima, *J. Chromatogr. B* **1998**, *712*, 105.
2) O. Al-Dirbashi, N. Kuroda, F. Menichini, S. Noda, M. Minemoto, K. Nakashima, *Analyst* **1998**, *123*, 2333.
3) O. Y. Al-Dirbashi, N. Kuroda, M. Wada, M. Takahashi, K. Nakashima, *Biomed. Chromatogr.* **2000**, *14*, 293.
4) K. Nakashima, S. Kinoshita, M. Wada, N. Kuroda, W. R. G. Baeyens, *Analyst* **1998**, *123*, 2281.
5) M. Wada, S. Kinoshita, Y. Itayama, N. Kuroda, K. Nakashima, *J. Chromatogr. B* **1999**, *721*, 179.

Chromatogram of amines as 4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzoyl compounds



Column : Daisopak SP-120-5-ODS-BP
Column Size : 4.6 mm I.D.×250 mm
Mobile Phase : CH_3CN / H_2O = 65 / 35
Detection : Fluorescence λex 325 nm
λem 430 nm
Flow Rate : 1 mL / min

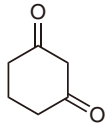
1. Phentermine
2. Fenfluramine

Labeling Reagent for Fluorescence Detection

of Carbonyl Compounds

1,3-Cyclohexanedione

5g [A5581]



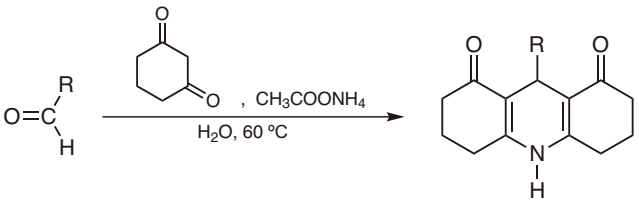
[A5581]

The compound **A5581** is an HPLC fluorescence labeling reagent, and can easily react with a carbonyl groups to form the corresponding decahydroacridine-1,8-dion (DHA) derivative. The resultant derivative is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 366 nm and 440 nm, respectively.

Application example:

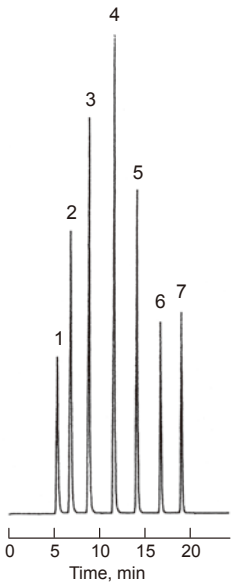
[Aliphatic aldehydes] [1,2)]

5 mL of acetic acid and 10 g of ammonium acetate are dissolved in distilled water. Then 0.25 g of labeling reagent **A5581** is added to the solution and shaken to prepare the derivatization reagent solution. 2 mL of this solution is added to 1 mL of aqueous solution (ethanol solution, in the case of long-chain aldehydes) containing 10-30 ng of an aliphatic aldehyde, and incubate at 60 °C for 30 min. After cooling, use 1 µL of this solution as an HPLC sample.



References 1) W. L. Stahovec, K. Mopper, *J. Chromatogr.* **1984**, *298*, 399.
2) Y. Suzuki, *Bunseki Kagaku* **1985**, *34*, 314.

Chromatogram of aldehydes as DHA derivatives



Column : Kaseisorb LC ODS-100-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3OH / H_2O = 40 / 60 → 90 / 10
20 min. linear gradient
Detection : Fluorescence λex 366 nm
λem 440 nm
Flow Rate : 1 mL / min

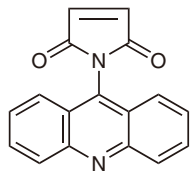
1. Formaldehyde
2. Acetaldehyde
3. Propionaldehyde
4. Butyraldehyde
5. Valeraldehyde
6. Hexylaldehyde
7. Heptylaldehyde2. Fenfluramine

Labeling Reagent for Fluorescence Detection

of Thiols

NAM [= *N*-(9-Acridinyl)maleimide]

50mg / 100mg [A5591]



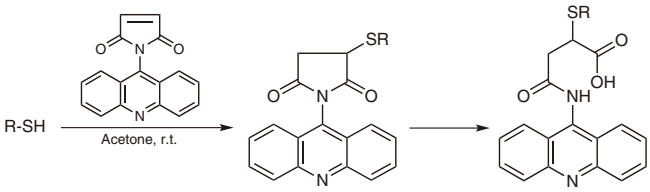
[A5591]

The compound **A5591** is an HPLC fluorescence labeling reagent, and can easily react with a mercapto group at room temperature. The resultant sulfide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 355 nm and 465 nm, respectively.

Application example:

[Thiols] [1-5]

0.4 mL of 30% NaOH solution and 1 mL of 0.2 M boric acid buffer solution (pH 8.8) are added to 2 mL of 1 mM sample solution in water. To this solution, 0.5 mL of 10 mM labeling reagent **A5591** / acetone solution is added and shaken, and reacted at room temperature for 30 min to use it as a HPLC sample.



References 1) Y. Nara, K. Tujimura, *Bunseki Kagaku* **1973**, *22*, 451.
2) Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1978**, *42*, 793.
3) H. Takahashi, Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1979**, *43*, 1439.
4) H. Takahashi, Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1976**, *40*, 2493.
5) H. Takahashi, T. Yoshida, H. Meguro, *Bunseki Kagaku* **1981**, *30*, 339.

Chromatogram of thiols as NAM derivatives

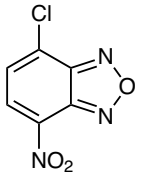
Column : Kaseisorb LC ODS-300-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : 0.05 M Na_2HPO_4 / CH_3CN = 89 / 11 (pH 7.5)
Detection : Fluorescence λex 355 nm
λem 465 nm
Flow Rate : 1 mL / min

1. *N*-Acetyl-L-cysteine
2. 2-Mercaptoethanol

Labeling Reagent for Fluorescence Detection

of Amines and Thiols

NBD-Cl [= 4-Chloro-7-nitro-2,1,3-benzoxadiazole] 1g / 5g [A5592]



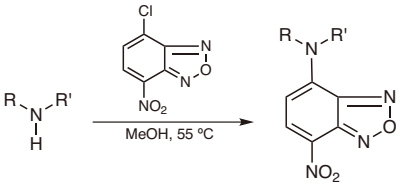
[A5592]

The compound **A5592** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with a secondary amine and thiol. The resultant derivative is stable enough to reach the detector without any decomposition under general reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 460 nm and 535 nm, respectively.

Application example:

[Alkylamines] [1)]

To 25-500 µL of a methanol solution containing an amine (1-20 µg), 4-8 eq. excess amount of 0.05% labeling reagent **A5592** / methanol solution is added. Af ter adding 50-100 µL of 0.1 M $NaHCO_3$, incubate at 55 °C for 1-5 h. After cooling the reaction mixture to room temperature, use it as an HPLC sample.

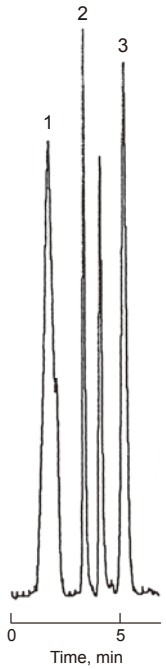


[Others]

TLC and HPLC of *N*-methylcarbamates, *N*,*N*-dimethylcarbamates in agrichemicals[2,3)]
Hydrolyze the carbamates to label the amine derivatives.
TLC of amphetamines in urine,[4,5)] HPLC of prolines (precolumn derivatization method)[6)]

References 1) H.-J. Klimisch, L. Stadler, *J. Chromatogr.* **1974**, *90*, 141.
2) J. F. Lawrence, R. W. Frei, *Anal. Chem.* **1972**, *44*, 2046.
3) R. W. Frei, J. F. Lawrence, *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 1259.
4) J. Monforte, R. J. Bath, I. Sunshine, *Clin. Chem.* **1972**, *18*, 1329.
5) F. van Hoof, A. Heyndrickx, *Anal. Chem.* **1974**, *46*, 286.
6) J. H. Wolfram, *J. Chromatogr.* **1977**, *132*, 37.
7) Y. Nishikawa, K. Kuwata, *Anal. Chem.* **1985**, *57*, 1864.

Chromatogram of alkylamines as NBD derivatives



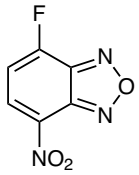
Column : Kaseisorb LC ODS-300-5
Column Size : 4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 45 / 55
Detection : Fluorescence λex 460 nm
λem 535 nm
Flow Rate : 1 mL / min

1. Propylamine
2. Butylamine
3. Amylamine

Labeling Reagent for Fluorescence Detection

of Amines and Thiols

NBD-F [= 4-Fluoro-7-nitro-2,1,3-benzoxadiazole] 100mg [A5593]



[A5593]

The compound **A5593** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with amino or mercapto groups to form the corresponding derivatives. **A5593** itself does not fluoresce, and its ethanol solution is relatively stable for a week in a refrigerator. The derivatives can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 530 nm, respectively.

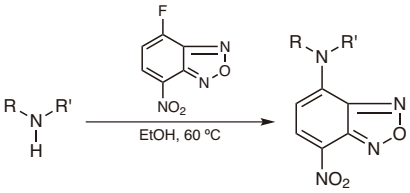
Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. Thus, further highly sensitive detection can be done by using laser induced fluorescence detector. When the reagent is hydrolyzed (NBD-OH), its fluorescence can be erased under an acidic condition. Therefore, this hydrolyzed reagent can be used as a post column reaction reagent.[5,7)]

Application example:

[Amino acids] [2,3)]

To 10 µL of 50 µM amino acid standard solution, add 10 µL of 0.1 M boric acid buffer solution (pH 8.0) and 20 µL of 50 mM labeling reagent **A5593** in ethanol solution, and incubate the mixture at 60 °C for 1 min. Immediately cool it with ice bath, and add 460 µL of 5 mM HCl to the reactant solution.

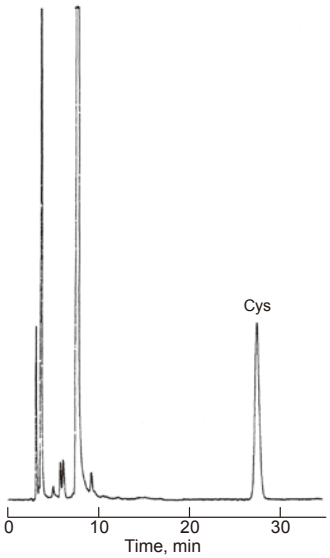
Use 10 µL of the solution as an HPLC sample.



References
1) K. Imai, Y. Watanabe, *Anal. Chim. Acta* **1981**, *130*, 377.
2) Y. Watanabe, K. Imai, *Anal. Biochem.* **1981**, *116*, 471.
3) Y. Watanabe, K. Imai, *J. Chromatogr.* **1982**, *239*, 723.
4) T. Toyo'oka, Y. Watanabe, K. Imai, *Anal. Chim. Acta* **1983**, *149*, 305.
5) Y. Watanabe, K. Imai, *Anal. Chem.* **1983**, *55*, 1786.
6) Y. Watanabe, K. Imai, *J. Chromatogr.* **1984**, *309*, 279.
7) H. Miyano, T. Toyo'oka, K. Imai, *Anal. Chim. Acta* **1985**, *170*, 81.
8) H. Kotaniguchi, M. Kawakatsu, T. Toyo'oka, K. Imai, *J. Chromatogr.* **1987**, *420*, 141.

Chromatogram of amino acids as NBD derivatives

Mobile Phase:
CH_3OH / THF / 0.1 M Phosphate buffer (pH 6.0)
= 20 / 20 / 60



Column : Kaseisorb LC ODS Super
Column Size : 4.6 mm I.D.×250 mm
Mobile Phase : CH_3OH / THF / 0.1 M
Phosphate buffer (pH 6.0) = 10 / 10 / 80
Temperature : 40 °C
Detection : Fluorescence λex 470 nm
λem 530 nm
Flow Rate : 1 mL / min

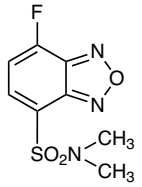
Mobile Phase:
CH_3OH / THF / 0.1 M Phosphate buffer (pH 6.0)
= 10 / 10 / 80

Labeling Reagent for Fluorescence Detection

of Amines and Thiols

DBD-F 100mg [A5595]

[= 4-(*N*,*N*-Dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole]



[A5595]

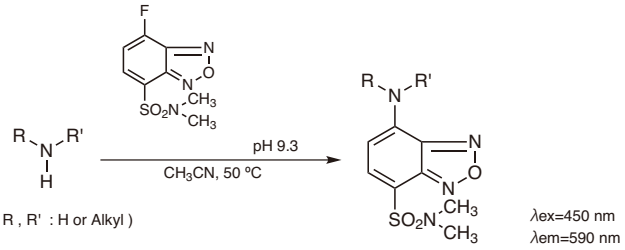
The compound **A5595** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with amino and mercapto groups to form the corresponding derivatives. The derivatives are stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 590 nm, respectively.

Application example:

[Amino acids]

0.5 mL of 20 mM labeling reagent **A5595** in acetonitrile is put into an amber-glass vial. To this solution, add 0.5 mL of 0.1 M boric acid buffer solution (pH 9.3, containing 1mM $EDTANa_2$) containing several nmol of an amino acid, and incubate at 50 °C for 30 min. After cooling the reaction mixture with ice bath, use it as an HPLC sample.

For example, the detection limit (S/N=3) for proline is 0.11 pmol.



(R , R' : H or Alkyl)

λex=450 nm
λem=590 nm

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Chromatogram of amino acids as DBD-amino acids

Column : Kaseisorb LC ODS-120-5
Column Size : 4.6 mm I.D.×250 mm
Mobile Phase : CH_3CN / H_2O / CH_3COOH = 50 / 50 / 1
Detection : Fluorescence λex 450 nm
λem 590 nm
Flow Rate : 1 mL / min

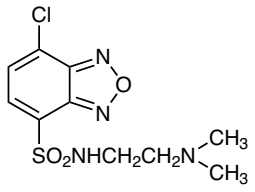
1. Valine
2. Leucine

Labeling Reagent for LC-MS/MS Analysis

of Proteins

DAABD-Cl 100mg [A5596]

[= 4-[2-(Dimethylamino)ethylaminosulfonyl]-7-chloro-2,1,3-benzoxadiazole]

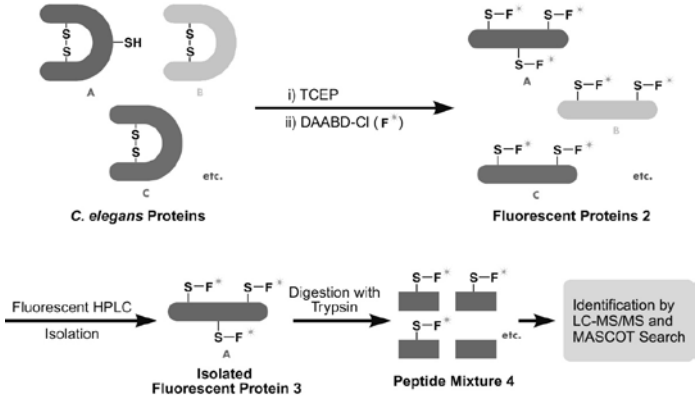


[A5596]

The relationship between genes and diseases has been studied extensively since the completion of human genome project in 2003. The direct cause of these diseases is sometimes related to the proteins produced in the human body by the human genome. The study of these proteins, “proteomics”, is very important in order to understand the relationship between genes and diseases.

The general method for protein analysis is isolation of the targeted protein by 2-D gel electrophoresis, followed by digestion with proteases to yield peptide fragment mixtures, which are then analyzed by MS/MS to identify the fragments, from which the isolated protein can then be reconstructed. However several problems still remain with 2-D gel electrophoresis, as extremely acidic, basic, or hydrophobic proteins cannot be fully separated. Furthermore, only the highly skilled experts are able to manage the 2-D gel electrophoresis to obtain reproducible data. For these reasons, new and improved methods for protein analysis have been explored.

Imai and co-workers have developed a new method for protein analysis with use of DAABD-Cl (**A5596**). This new method can analyze proteins with high precision. Imai and co-workers extracted proteins from breast cancer cells, and the extracted proteins were first reacted with tris(2-carboxyethyl)phosphine in a buffer solution in order to reductively cleave the S-S bonds to yield the primary proteins. The resulting SH functional groups of resulting proteins were derivatized by reaction with DAABD-Cl to yield fluorescent labeled protein mixtures (**2** in Scheme 1). The fluorescent labeled protein mixtures were separated by fluorescence HPLC to obtain fractions consisting of DAABD labeled proteins (Figure 1). The selected DAABD labeled protein (**3** in Scheme 1) was isolated and digested using trypsin to obtain the peptide mixtures (**4** in Scheme 1) consisting of DAABD labeled peptides and other peptides. The peptide mixtures were analyzed by LC-MS/MS and the resulting mass spectral data were analyzed to identify the original protein by the MASCOT database system (Scheme 1).



Scheme 1. Quantification and Identification of Expressed Proteins in cell with DAABD-Cl

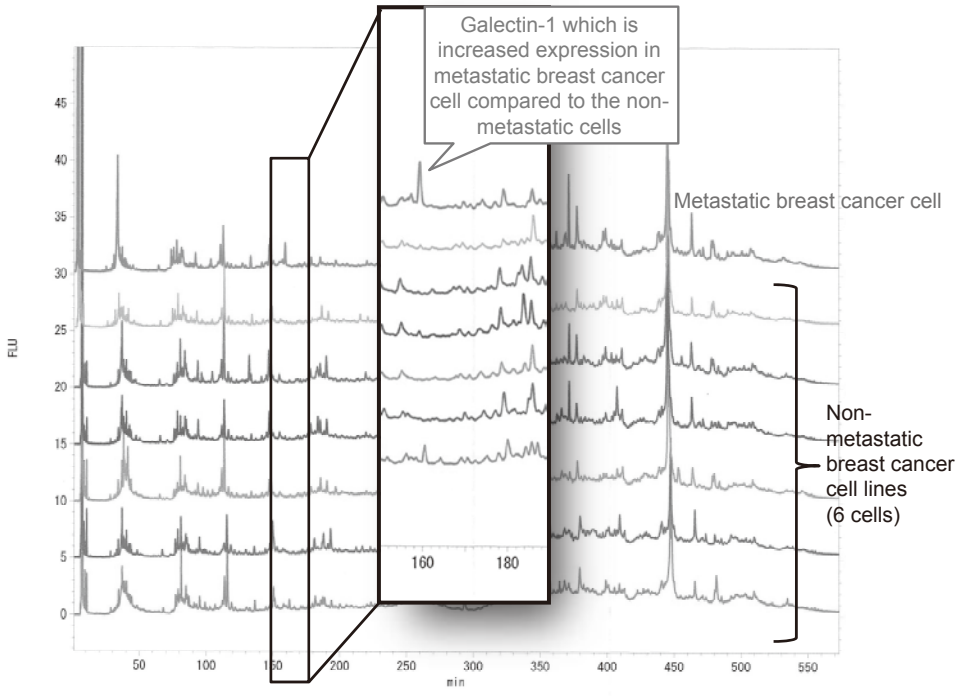


Figure 1. Chromatograms of the proteins in soluble fraction of breast cancer cells derivatized with DAABD-Cl

The chlorine at 7 position of DAABD-Cl reacts specifically with SH groups. DAABD-Cl itself is non-fluorescent, however the resultant DAABD-derivative is strongly fluorescent, due to the benzoxadiazole skeleton coupled to the SH group. Generally, there are not many S-S bonds and SH group in proteins, and consequently target proteins can be labeled with DAABD-Cl in an efficient manner. Additionally, both excitation and emission wavelengths of DAABD derivatives are long, allowing highly sensitive and selective protein analysis. Furthermore, DAABD-Cl has a dimethylamino group at 4 position, and therefore high intensity cations can be obtained with electron spray ionization during MS analysis. Therefore, extremely small quantities of peptides can be analyzed.

DAABD-Cl is a labeling reagent, which can effectively permit the collection of the target protein through fluorescence HPLC and analysis by MS/MS. This protein analysis reagent that Imai and co-worker have developed allows one to identify a very small amount of protein with good precision. It is expected that this technique (FD-LC-MS/MS method) can be used in many applications, including the identification of abnormal or pathogenic proteins in living organism.

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