

Separation of Bioactive Natural Products using Synthetic Adsorbents

 MITSUBISHI CHEMICAL CORPORATION

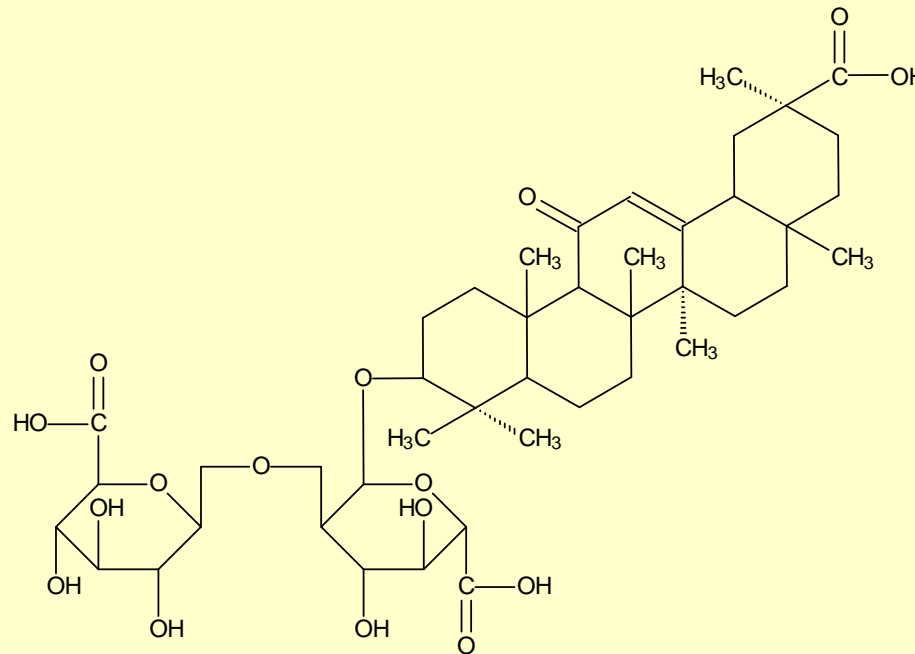
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Example of Application

- Glycyrrhizic Acid
- Taxol
- Curcumins
- Vitamins
- Tea extract, catechins
- Soybean Extract and Isoflavones
- Herbal Drugs
- Saponins
- Persimmon Tannins
- Carotenoide

Separation of a Herbal Drug: Glycyrrhizic Acid

- Structure of Glycyrrhizic Acid (Mw: 823)



Analytical separation of glycyrrhizic acid on polystyrenic and polymethacrylic adsorbents

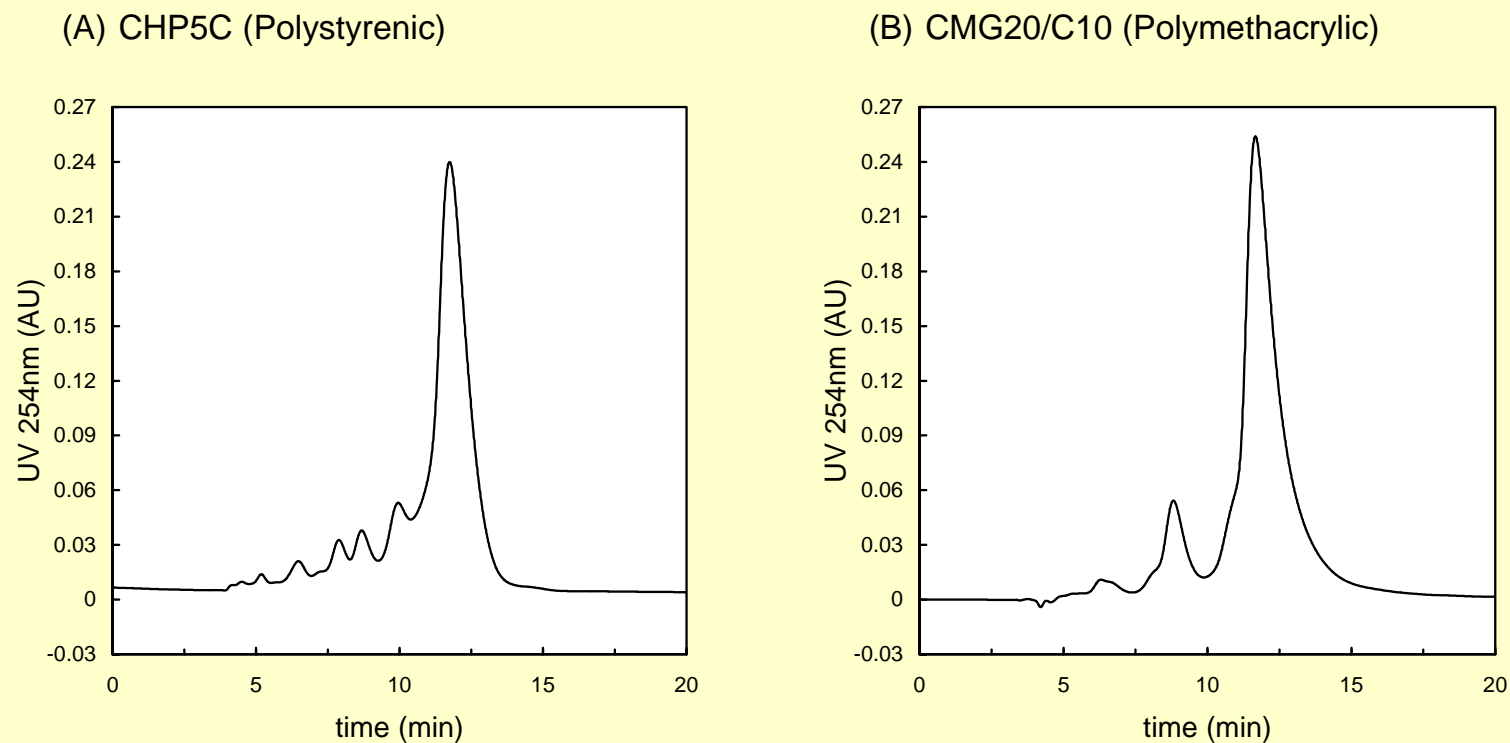


Figure. Separation of glycyrrhizic acid on polystyrenic and polymethacrylic adsorbents.

Conditions:

(A) Adsorbent, CHP5C (10 μ m); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/20mM Citrate (pH4.0)=65/35; Flow rate, 0.5ml/min.

(B) Adsorbent, CMG20/C10 (10 μ m); Column size, 150mm x 4.6mm I.D.;
Eluent, MeOH/20mM Citrate (pH4.0)=55/45; Flow rate, 0.5ml/min.

Sample: Glycyrrhizic Acid (Sigma G-2137, 2mg/ml). Injection: 10 μ l.

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Note: CHP5C is abolished and substitute is CHP20/C10.

Analytical Separation of Taxol on a Polystyrenic Adsorbent

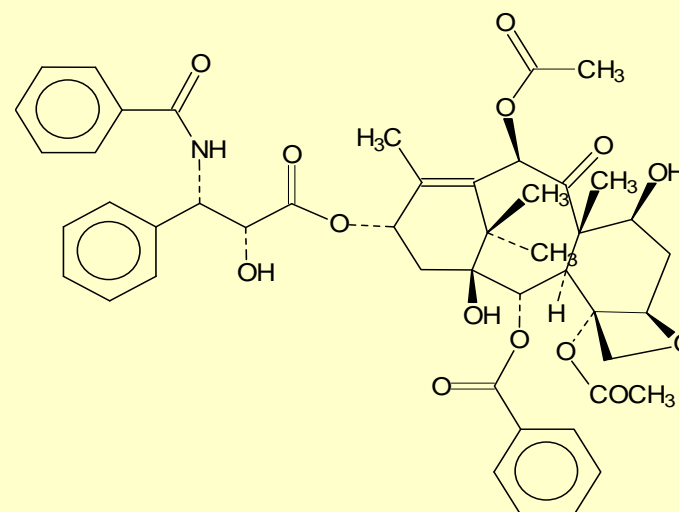
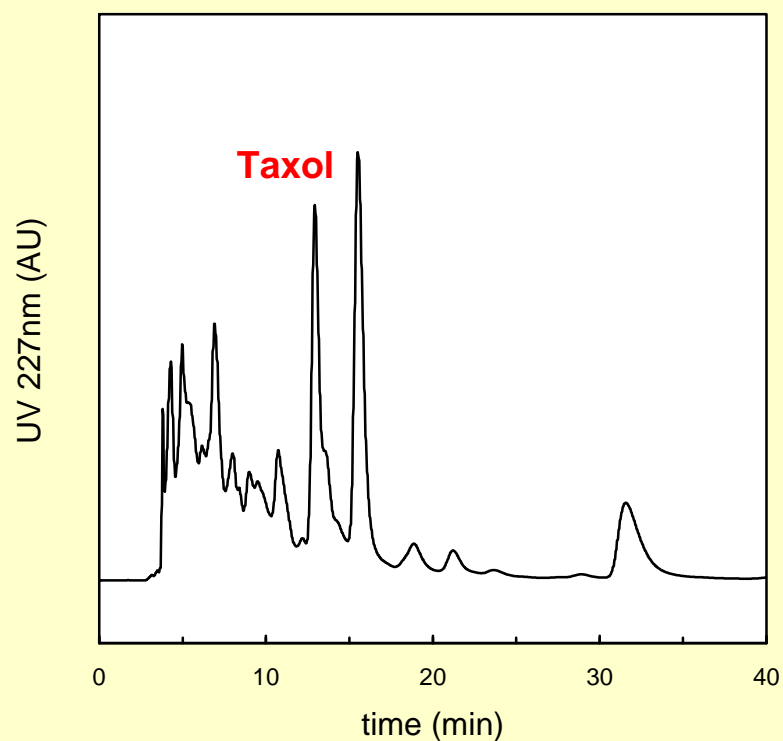
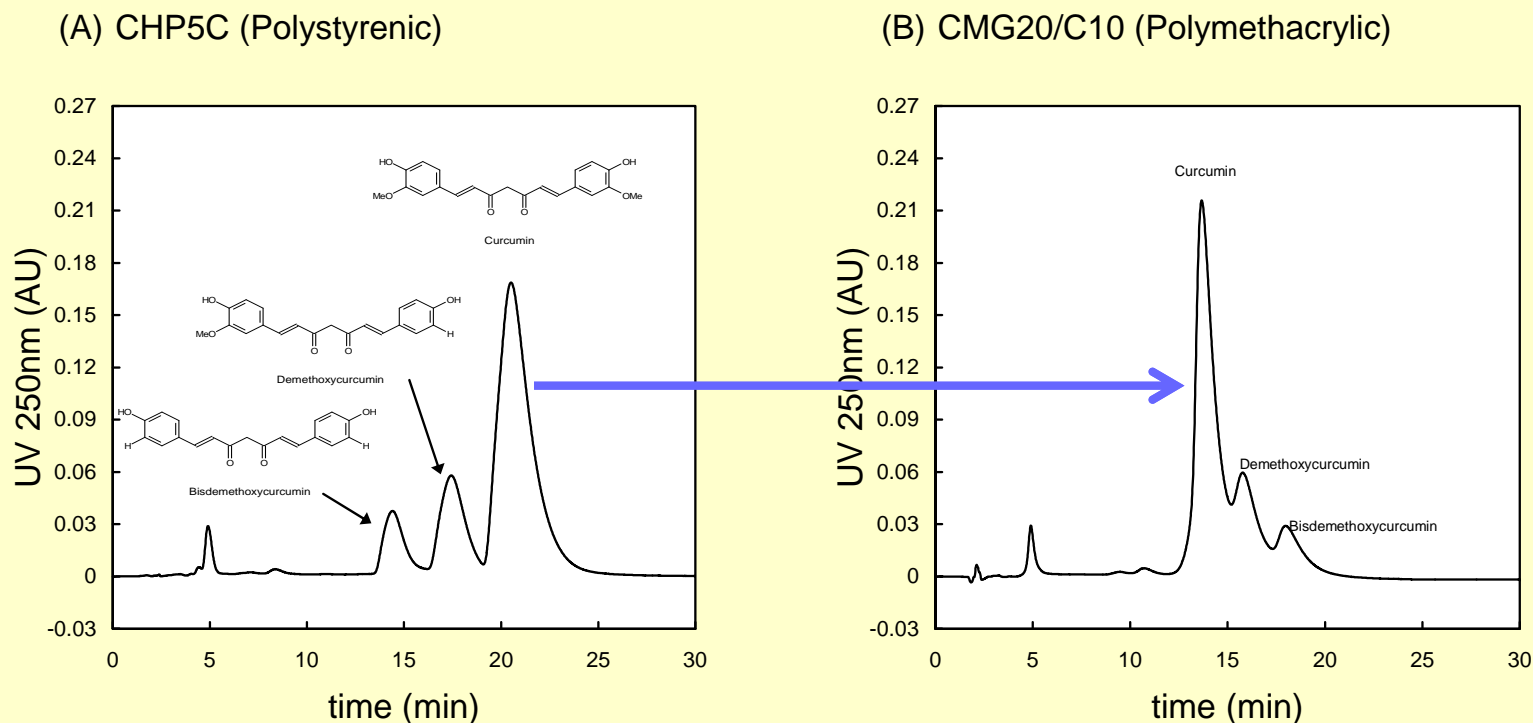


Figure. Separation of taxol on a polystyrenic adsorbent.

Conditions:

Adsorbent, CHP20/C04 (4 μ m); Column size, 150mm x 4.6mm I.D.;
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Eluent, AcCN/H₂O=60/40; Flow rate, 0.5ml/min.

Analytical separation of curcumins on polystyrenic and polymethacrylic adsorbents



Elution order is reversed.

Figure.

Separation of curcumins on polystyrenic and polymethacrylic adsorbents.

Conditions:

(A) Adsorbent, CHP5C (10 μ m); Column size, 150mm x 4.6mm I.D.;

(B) Adsorbent, CMG20/C10 (10 μ m); Column size, 150mm x 4.6mm I.D.;

Eluent, AcCN/0.04M phosphoric acid=50/50; Flow rate, 0.46ml/min.

Sample: Curcumin (Sigma C-1386, 1mg/ml). Injection: 12.5 μ l.

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Note: CHP5C is abolished and substitute is CHP20/C10.

Analytical Separation of Vitamins on a Polymethacrylic Adsorbent

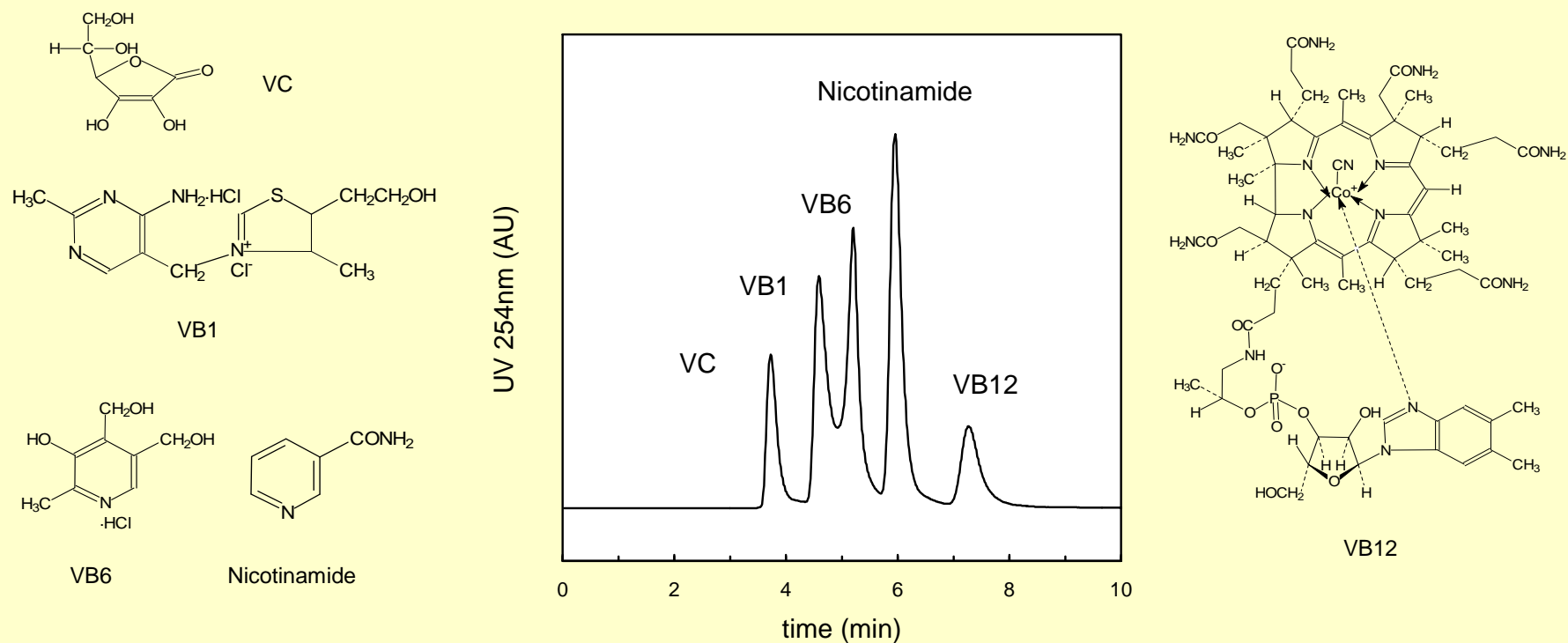


Figure. Separation of water-soluble vitamins on a polymethacrylic adsorbent.

Conditions:

Adsorbent, CMG20/C04 (4 μ m); Column size, 150mm x 4.6mm I.D.;

Eluent, 100mM Phosphate (pH 7.0) R90/19; Flow rate, 0.5ml/min.

Flow rate, 0.5ml/min; Temperature, 55°C.

Example of Scale-up Separation

- Separation of tea catechins
 - Optimization of elution conditions using CHP5C
(Abolished, substitute is CHP20/C10)
 - Semi-preparative separation using CHP50/P20, CHP50/P30
 - Preparative separation and fraction analysis
 - Purity and recovery of (-)-epigallocatechin gallate

Resin	Column size	Resin volume	Loadability	Loading amount	Purity	Recovery
CHP5C (10 μ m)	150 x 4.6mmI.D.	2.50ml	0.05g/L	125 μ g	-	-
CHP50/P20 (18 μ m)	465 x 32mmI.D.	374ml	0.50g/L	187mg	99%	82%
CHP50/P30 (30 μ m)	435 x 90mmI.D.	2770ml	1.01g/L	2800mg	99%	61%

Separation of tea extract on a column packed with analytical polystyrenic adsorbent

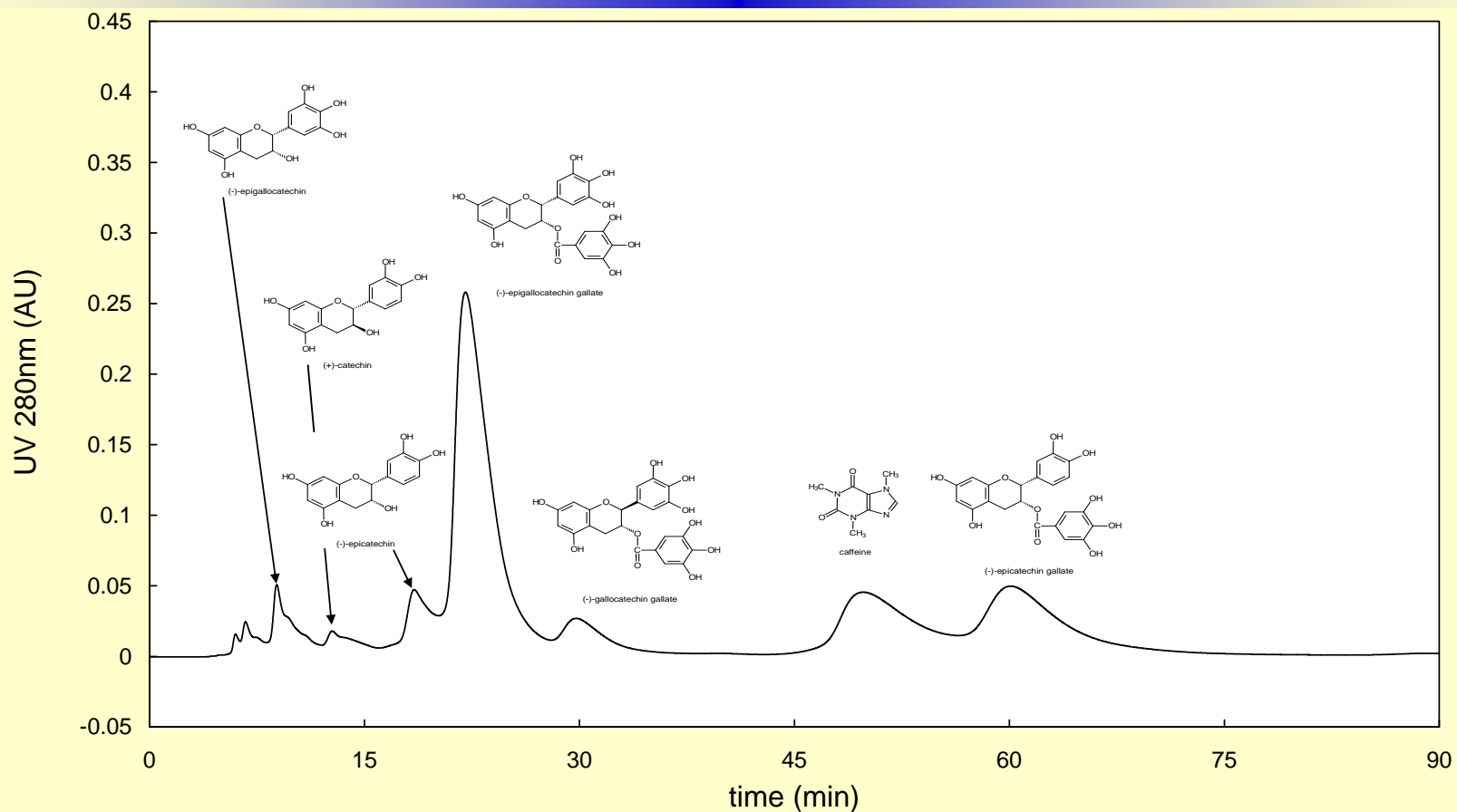


Figure. Separation of tea extract on a column packed with analytical polystyrenic adsorbent.

Conditions: Adsorbent, CHP5C (10 μ m); Column size, 150mm x 4.6mm I.D.;

Eluent, MeOH/0.01M Acetic acid=35/65; Flow rate, 0.46ml/min.

Sample: Polyphenol 60 (10mg/ml), Injections: 10 μ l.

Note: CHP5C is abolished and substitute is CHP20/C10.

Separation of tea extract on a column packed with analytical polymethacrylic adsorbent

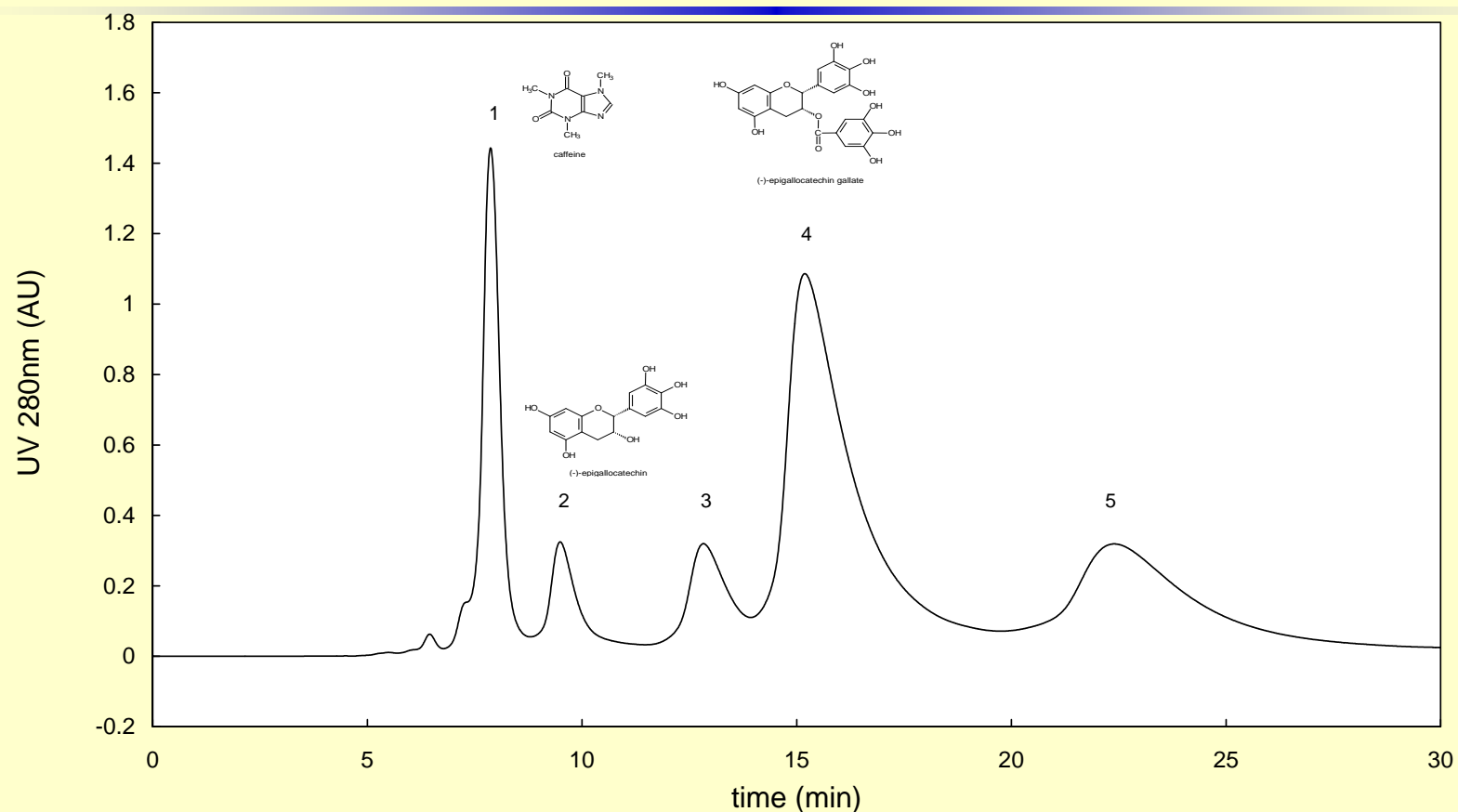


Figure. Separation of tea extract on a column packed with analytical polymethacrylic adsorbent of 10 μ m. Conditions: Adsorbent, CMG20/C10 (10 μ m); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.01M Acetic acid=60/40; Flow rate, 0.46ml/min.

Sample: Polyphenon.60 (10mg/ml). Injection: 12.5 μ l.

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Peak identification: 1= caffeine; 2=(-)-epigallocatechin; 3=(+)-catechin and (-)-epicatechin;
4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate and (-)-epicatechin gallate.

Chromatographic separation of tea extract on polystyrenic adsorbents with various particle sizes

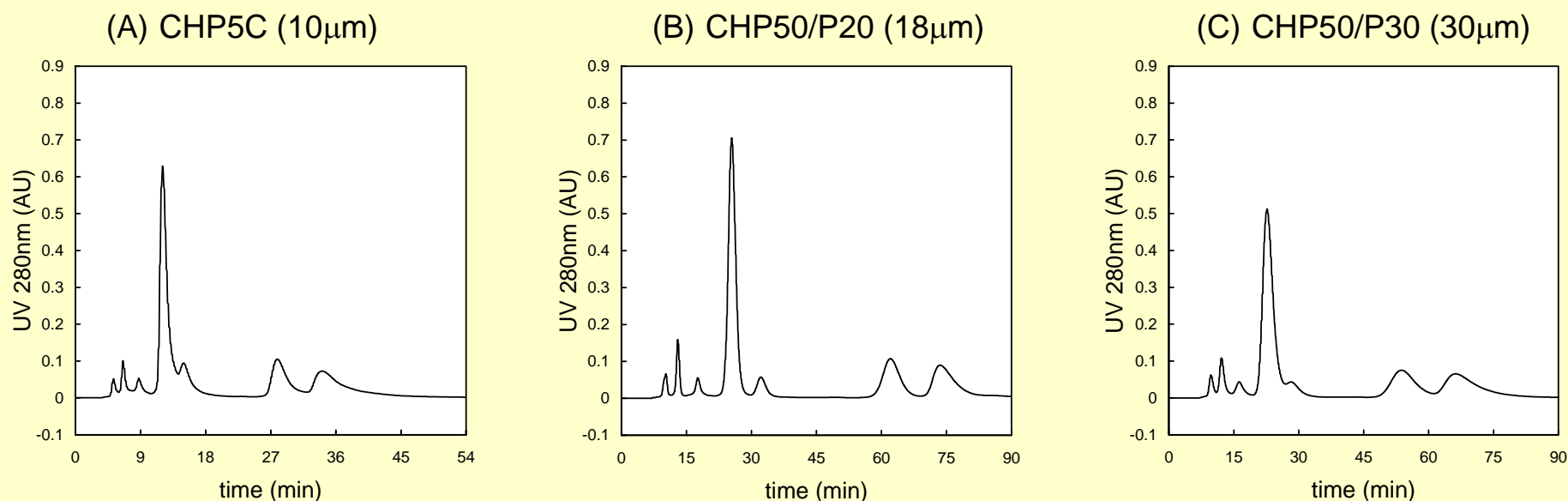


Figure 14. Chromatographic separation of tea extract on polystyrenic adsorbents with various particle sizes.

- (A) Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 0.46ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 10μl.
- (B) Adsorbent, CHP50/P20 (18μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 2.18ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 47μl.
- (C) Adsorbent, CHP50/P30 (30μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.01M Acetic acid=40/60; Flow rate, 2.18ml/min. Sample: Polyphenon 60 (10mg/ml). Injection: 47μl.

Note: CHP5C is abolished and substitute is CHP20/C10

Preparative separation of tea extract on polystyrenic adsorbent CHP50/P20

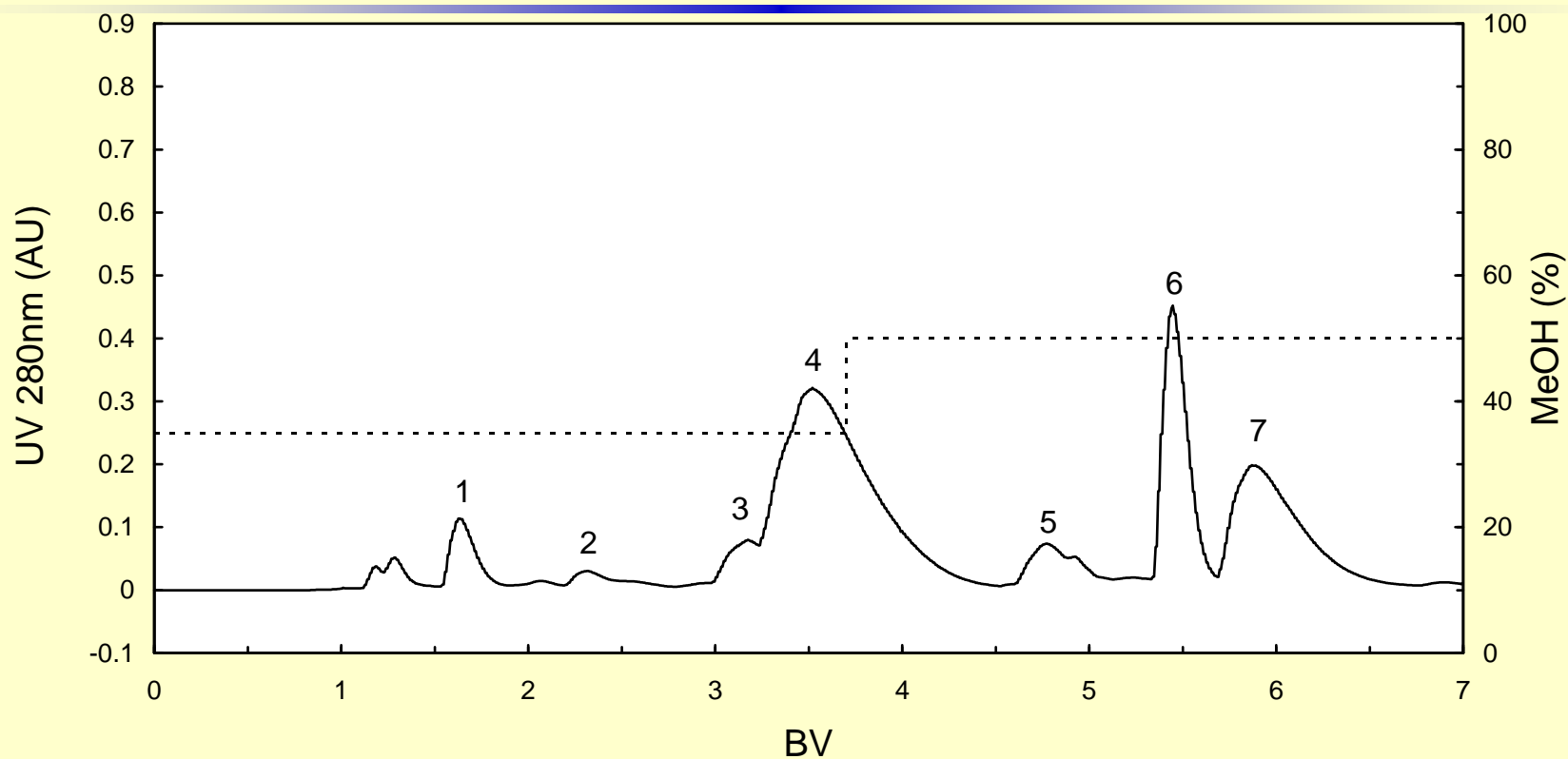


Figure. Preparative separation of tea extract on a column packed with polystyrenic adsorbent of 18 μ m.

Conditions:

Adsorbent, CHP50/P20 (18 μ m); Column size, 465mm x 32mm I.D. (374ml);

Eluent, 0-185min: MeOH/0.01M Acetic acid=35/65;

185-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 7.48ml/min (SV = 1.2).

Sample: Polyphenon 60 (10mg/ml). Injection: 18.7ml (0.05BV).

Peak identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin;

4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=(-)-epicatechin gallate; 7=caffeine.

Elution profile of each catechin derivative on polystyrenic adsorbent CHP50/P20

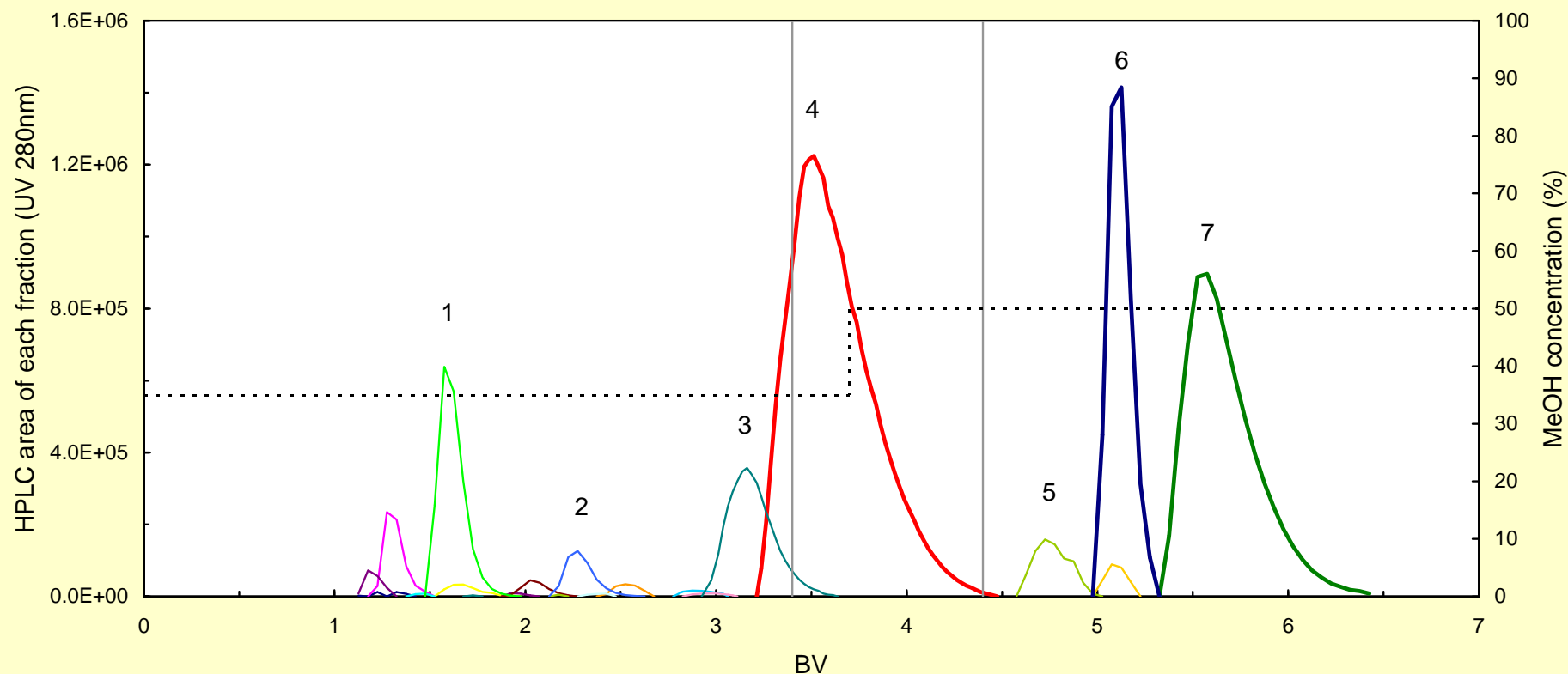


Figure. Elution profile of each catechin derivative determined by the fraction analysis.

Conditions: Adsorbent, CHP50/P20 (18 μ m); Column size, 465mm x 32mm I.D. (374ml); Eluent, 0-185min: MeOH/0.01M Acetic acid=35/65;185-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 7.48ml/min (SV = 1.2). Sample: Polyphenon 60 (10mg/ml). Injection: 18.7ml (0.05BV).

Identification: 1=(-)-epigallocatechin; 2=(+)-catechin; 3=(-)-epicatechin; 4=(-)-epigallocatechin gallate; 5=(+)-gallocatechin gallate; 6=caffeine; 7=(-)-epicatechin gallate.

Preparative separation of tea extract on polystyrenic adsorbent CHP50/P30

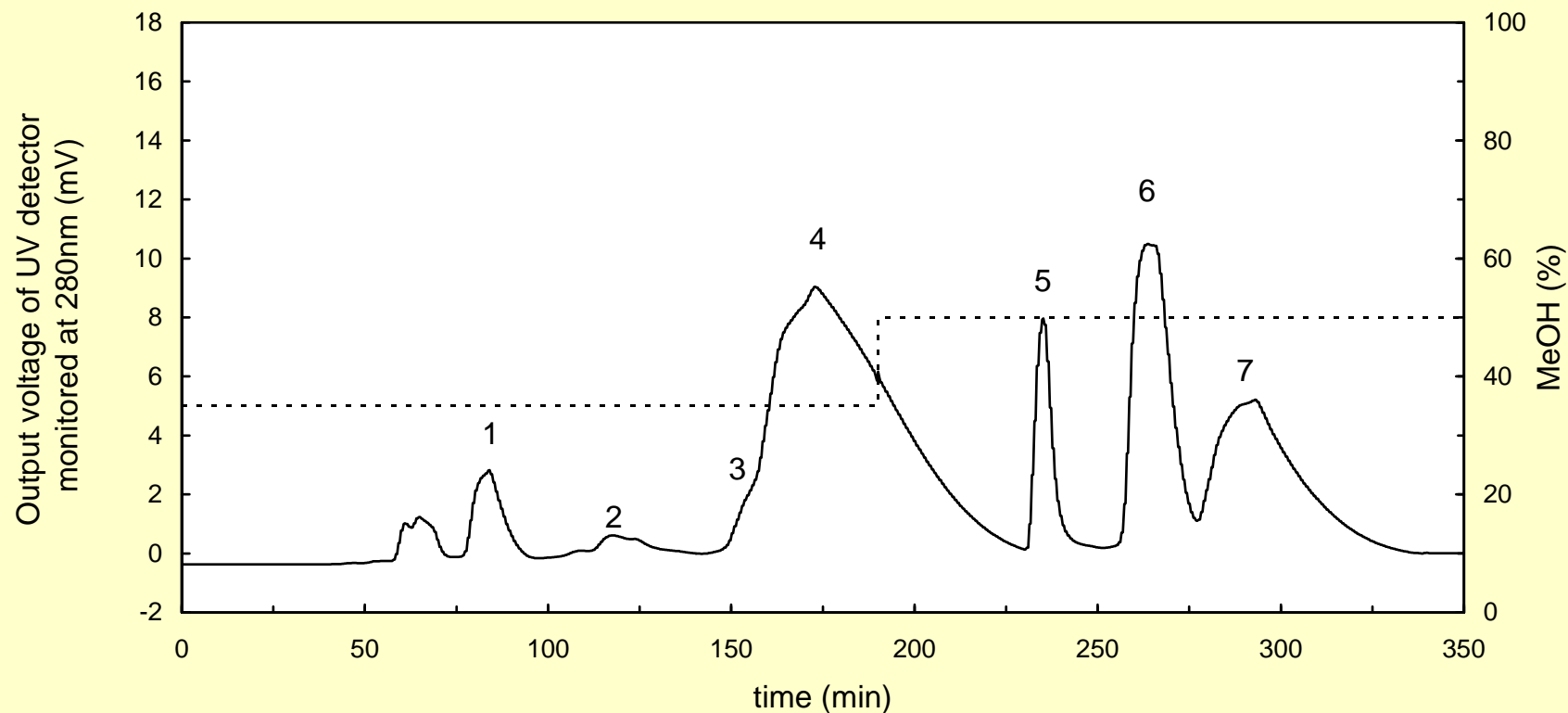


Figure. Scale-up preparative separation of tea extract on a column packed with polystyrenic adsorbent of 30 μ m.

Conditions:

Adsorbent, CHP50/P30 (30 μ m); Column size, 435mm x 90mm I.D.;

Eluent, 0-190min: MeOH/0.01M Acetic acid=35/65;

190-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 55ml/min.

Sample: Polyphenon 60 (20mg/ml). Injection: 140ml.

Peak identification: 1=Catechin; 2=Epigallocatechin; 3=Epigallocatechin gallate;

4=(-)-epigallocatechin gallate; 5=(-)-gallocatechin gallate; 6=(-)-epicatechin gallate; 7=caffeine.

Elution profile of each catechin derivative on polystyrenic adsorbent CHP50/P30

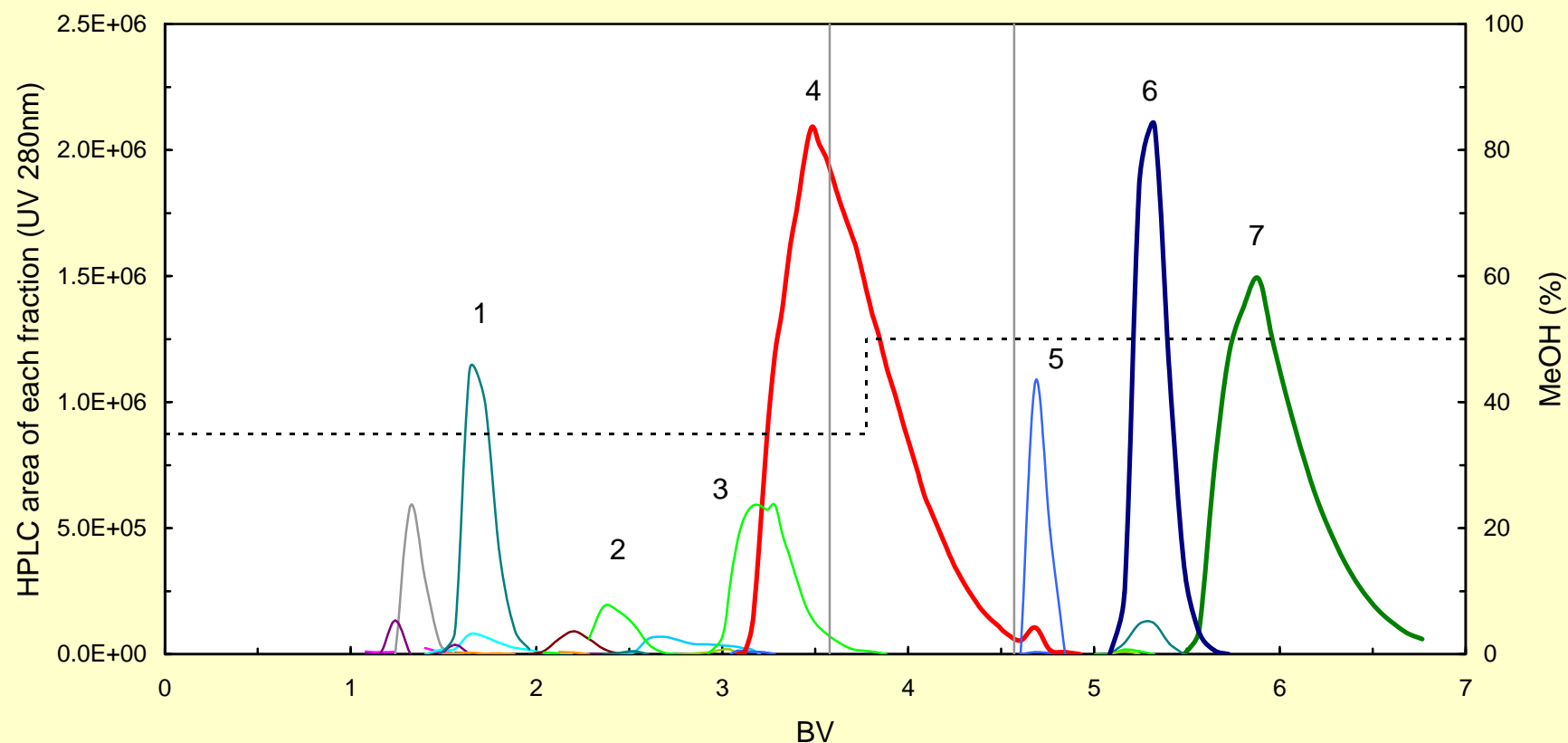


Figure. Elution profile of each catechin derivative determined by the fraction analysis.

Conditions: Adsorbent, CHP50/P30 (30 μ m); Column size, 435mm x 90mm I.D. (2770ml); Eluent, 0-190min: MeOH/0.01M Acetic acid=35/65; 190-350min: MeOH/0.01M Acetic acid=50/50; Flow rate, 55ml/min (SV = 1.2). Sample: Polyphenon 60 (20mg/ml).

Injection: 140ml (0.05BV).

Identification: 1=(-)-epigallocatechin, 2=(+)-catechin, 3=(-)-epicatechin, 4=(-)-epigallocatechin gallate, 5=(-)-gallocatechin gallate, 6=caffeine, 7=(-)-epicatechin gallate.

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Chromatograms of soybean crude extract and isoflavones on analytical polystyrenic adsorbent

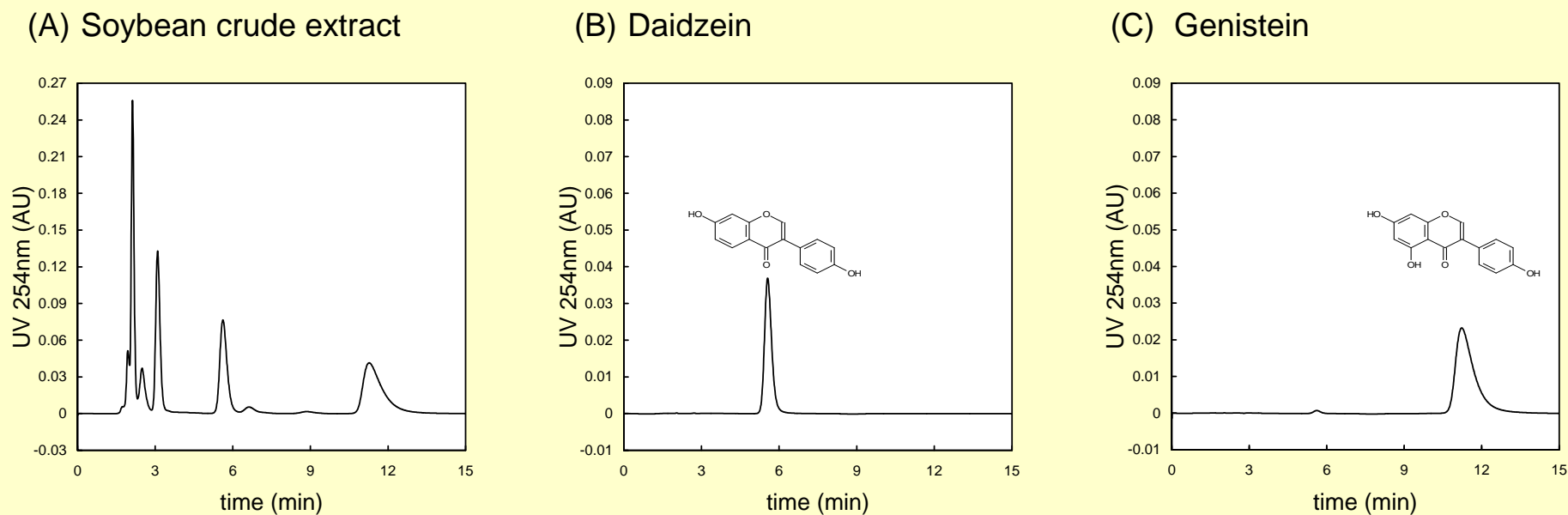


Figure. Chromatograms of soybean crude extract and isoflavones on analytical polystyrenic adsorbent.

Adsorbent, CHP5C (10 μ m); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 1.00ml/min.

(A) Sample: Soybean crude extract. Injection: 10.0 μ l.

(B) Sample: Daidzein (100 μ g/ml). Injection: 1.0 μ l.

(C) Sample: Genistein (100 μ g/ml). Injection: 1.0 μ l.

Note: CHP5C is abolished and substitute is CHP20/C10.

Separation of soybean crude extract on polystyrenic adsorbents with various particle sizes

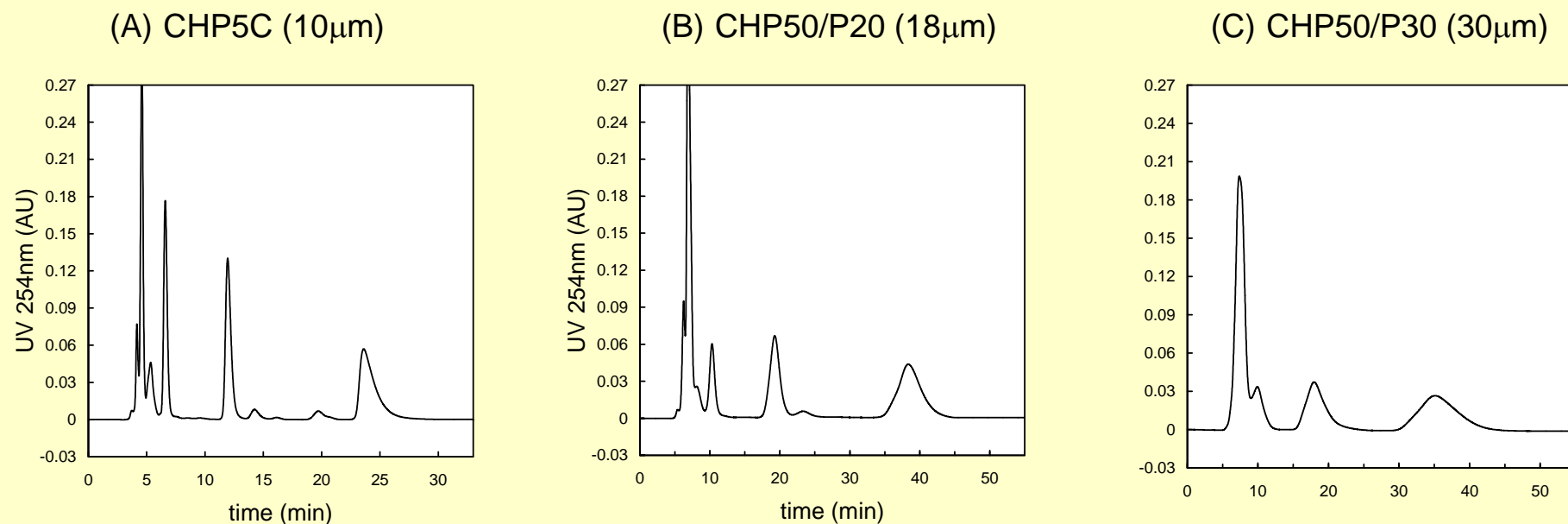


Figure. Chromatographic separation of soybean crude extract on polystyrenic adsorbents with various particle sizes.

- (A) Adsorbent, CHP5C (10μm); Column size, 150mm x 4.6mm I.D.; Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 0.46ml/min. Sample: Soybean crude extract. Injection: 12.5μl.
- (B) Adsorbent, CHP50/P20 (18μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 2.18ml/min. Sample: Soybean crude extract. Injection: 100μl.
- (C) Adsorbent, CHP50/P30 (30μm); Column size, 250mm x 10mm I.D.; Eluent, MeOH/0.1M ammonium acetate=80/20; Flow rate, 2.18ml/min. Sample: Soybean crude extract. Injection: 100μl.

Note: CHP5C is abolished and substitute is CHP20/C10.

Preparative separation of soybean crude extract on polystyrenic adsorbent CHP50/P20

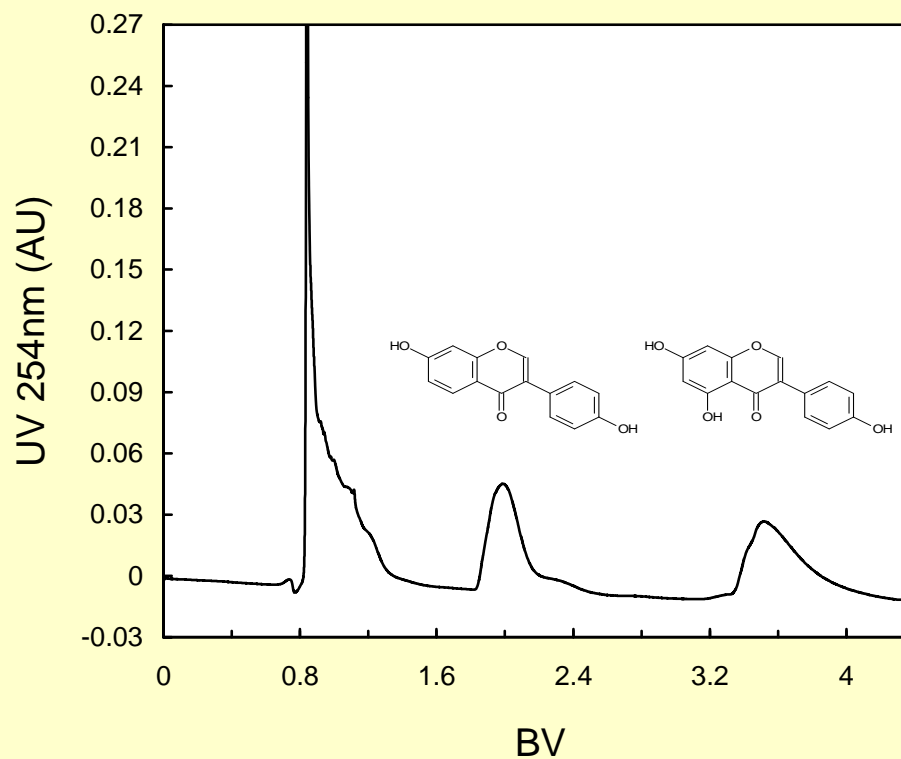


Figure. Preparative chromatographic separation of soybean crude extract on polystyrenic adsorbent with fine grade particle size of 18 μ m.

Conditions:

Adsorbent, CHP50/P20 (18 μ m); Column size, 465mm x 32mm I.D.;

Eluent, MeOH:0.1M ammonium acetate=80:20; Flow rate, 7.46ml/min.

Sample: Soybean crude extract. Injection: 37.4ml (0.1BV).

Applications from Patents – Separation of Herbal Drugs

- Examples of HP20
 - β -Glucuronidase inhibitor from “KEIGAI” used for antitumor agent
 - Jpn. Kokai Tokkyo Koho JP 05043469
(Tsumura and Co.; Yakult Honsha Co., Ltd., Japan)
 - Saikosaponin from “MISHIMASAIKO” (*Bupleurum falcatum* L.) for anti-inflammatory
 - Jpn. Kokai Tokkyo Koho JP 09067388 (Shiseido Co Ltd, Japan)
 - Pilocarpine from *Pilocarpus jaborandi* (Citrus) used for perspiration promoter
 - Jpn. Kokai Tokkyo Koho JP 09188628 (Tsumura and Co., Japan)
 - Mutation inhibitors comprising condensed tannins from *Diospyros Kaki* Thunb (persimmon)
 - Jpn. Kokai Tokkyo Koho JP 09315985 (Kanebo, Ltd., Japan)
 - Anti-HIV agent from *A. hypogaea* (peanut)
 - Jpn. Kokai Tokkyo Koho JP 11246431
(Agency of Industrial Sciences and Technology; Tokiwa Shokubutsu Kagaku Kenkyusho Ltd., Japan)

Applications from Patents – Separation of Herbal Drugs

- Example of HP20
 - Adsorption properties of lycopene from broth of *Blakeslea trispora* by macroporous resin
 - Zhongguo Shengwu Gongcheng Zazhi (2010), 30(4), 71-76
- Example of HP21
 - Food for tooth decay prevention from Oolong Tea
 - Jpn. Kokai Tokkyo Koho JP 04178320 (Suntory, Ltd., Japan)
 - Separation of phenylalanine and cinnamic acid
 - Jpn. Kokai Tokkyo Koho (1986), JP 61194056 A
- Example of HP2MGL
 - Method for production of saponins from *Cynanchum* by using methacrylic or aromatic adsorbents-
 - Jpn. Kokai Tokkyo Koho (2005), JP 2005289957 A

Applications from Patents – Separation of Herbal Drugs

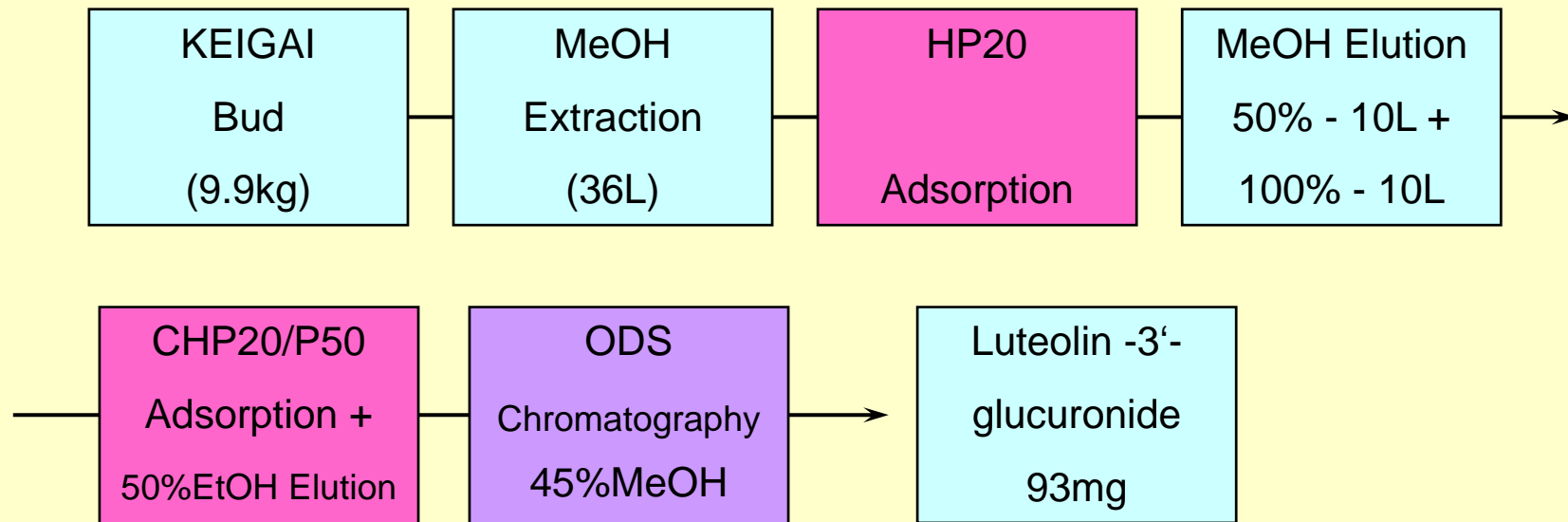
- Example of SP207
 - Xanthone glycosides for improvement of brain functions from “ENSHI”
- Jpn. Kokai Tokkyo Koho JP 07179487 (Tsumura & Co, Japan)
 - Extraction and purification of artemisinin from plant material
Faming Zhuanli Shenqing (2013), CN 103059039 A
 - Method for extraction and purification of tripterine
Faming Zhuanli Shenqing (2013), CN 103059094 A
 - Recovery and concentration of antioxidants from winery wastes
Molecules (2012), 17, 3008-3024
 - Method for extracting polyphenols from cinnamomum cassia and application of cinnamomum cassia polyphenol extract in treating non-insulin-dependent diabetes mellitus
Faming Zhuanli Shenqing (2011), CN 102274244 A
 - Deodorization of Soybean Proteins by Enzymatic and Physicochemical Treatments (hexanal removal)
Journal of Agricultural and Food Chemistry (2002), 50(6), 1652-1658
 - Blood sucrose-reducing chinese medicinal composition containing cinnamon polyphenol and its preparation method
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Faming Zhuanli Shenqing (2012), CN 102319289 A

Applications from Patents – Separation of Herbal Drugs

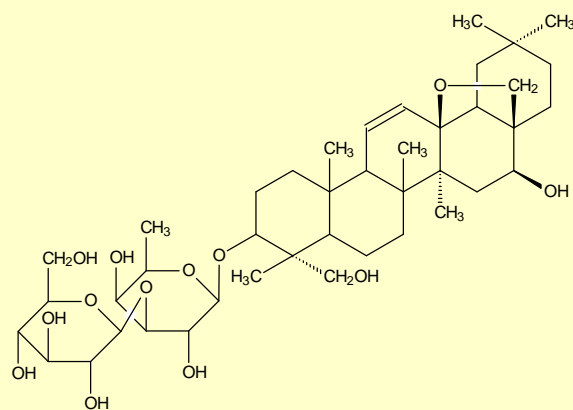
- Example of SP825
 - Optimization of sulforaphane separation from broccoli seeds by macroporous resins –
Separation Science and Technology (2008), 43(3), 609-623
 - Method for preparing tobacco essence containing Aloe extract
Faming Zhuanli Shenqing (2013), CN 103045370 A
 - Separation of injectable salidroside by column chromatography of macroporous resins for treating myocardial ischemia
Science China: Chemistry (2012), 55(7), 1435-1444
- Example of HP20SS
 - Comparative Study of Chromatographic Medium-Associated Mass and Potential Antitumor Activity Loss with Bioactive Extracts Journal of Natural Products (2013), 76(4), 642-647

Applications from Patents – Separation of Herbal Drugs

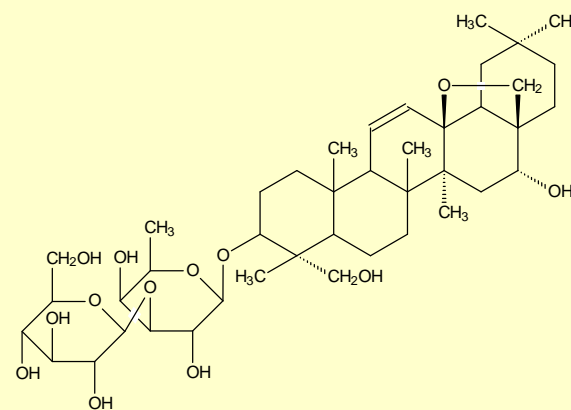
- β -Glucuronidase inhibitor from “KEIGAI” used for antitumor agent
- Jpn. Kokai Tokkyo Koho JP 05043469
(Tsumura and Co.; Yakult Honsha Co., Ltd., Japan)



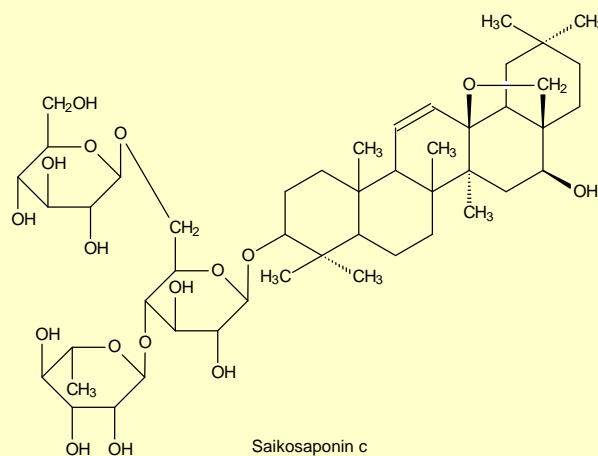
Applications from Patents – Separation of Saikosaponins



Saikosaponin a



Saikosaponin d

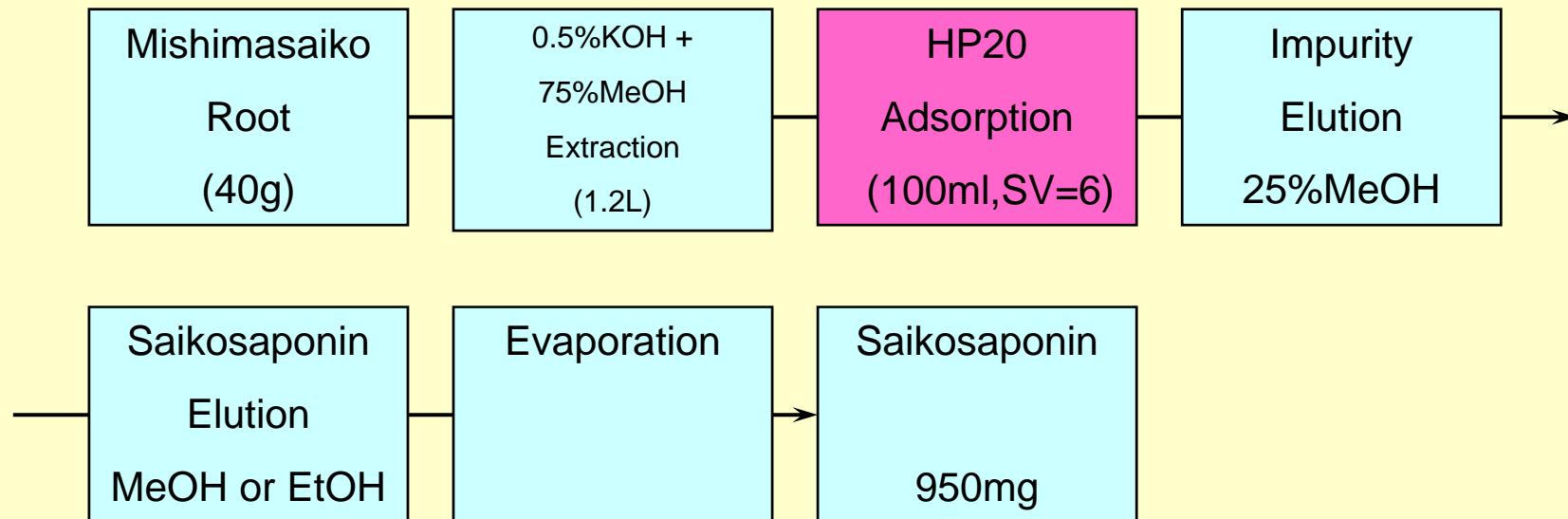


Saikosaponin c

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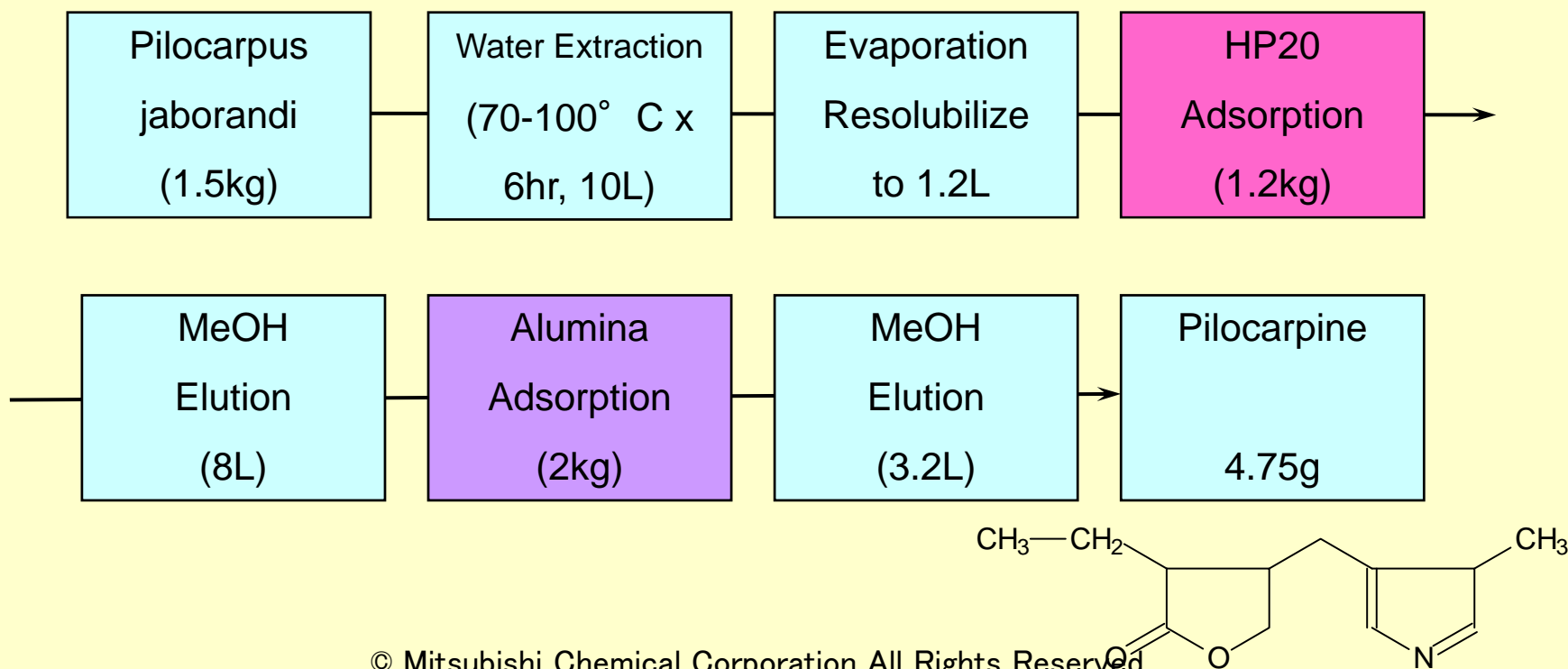
Applications from Patents – Separation of Herbal Drugs

- Saikosaponin from “MISHIMASAIKO” (Bupleurum falcatum L.) for anti-inflammatory
 - Jpn. Kokai Tokkyo Koho JP 09067388 (Shiseido Co Ltd, Japan)

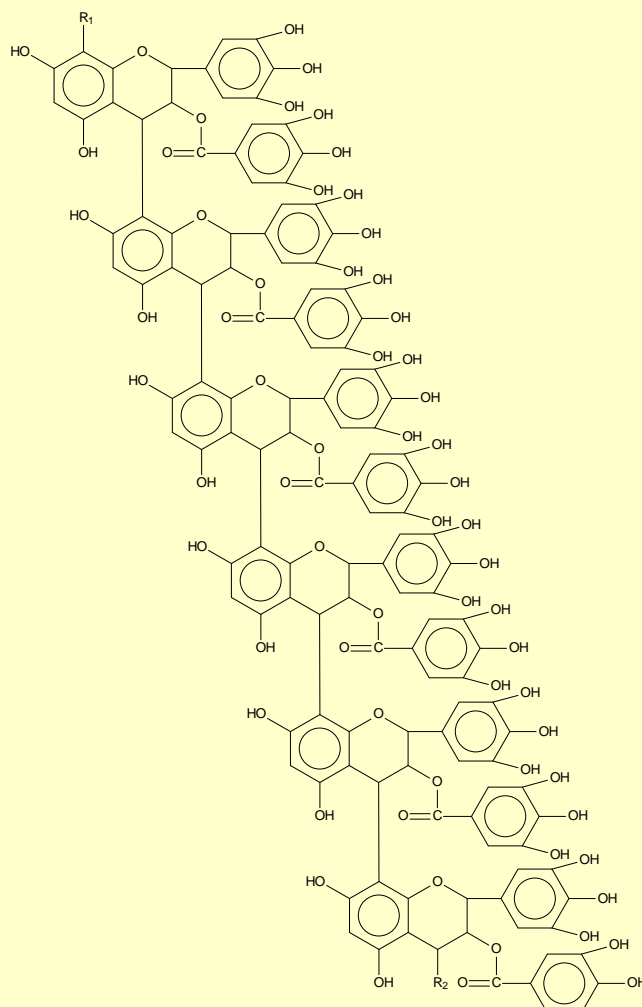


Applications from Patents – Separation of Herbal Drugs

- Pilocarpine from *Pilocarpus jaborandi* (Citrus) used for perspiration promoter
 - Jpn. Kokai Tokkyo Koho JP 09188628 (Tsumura and Co., Japan)



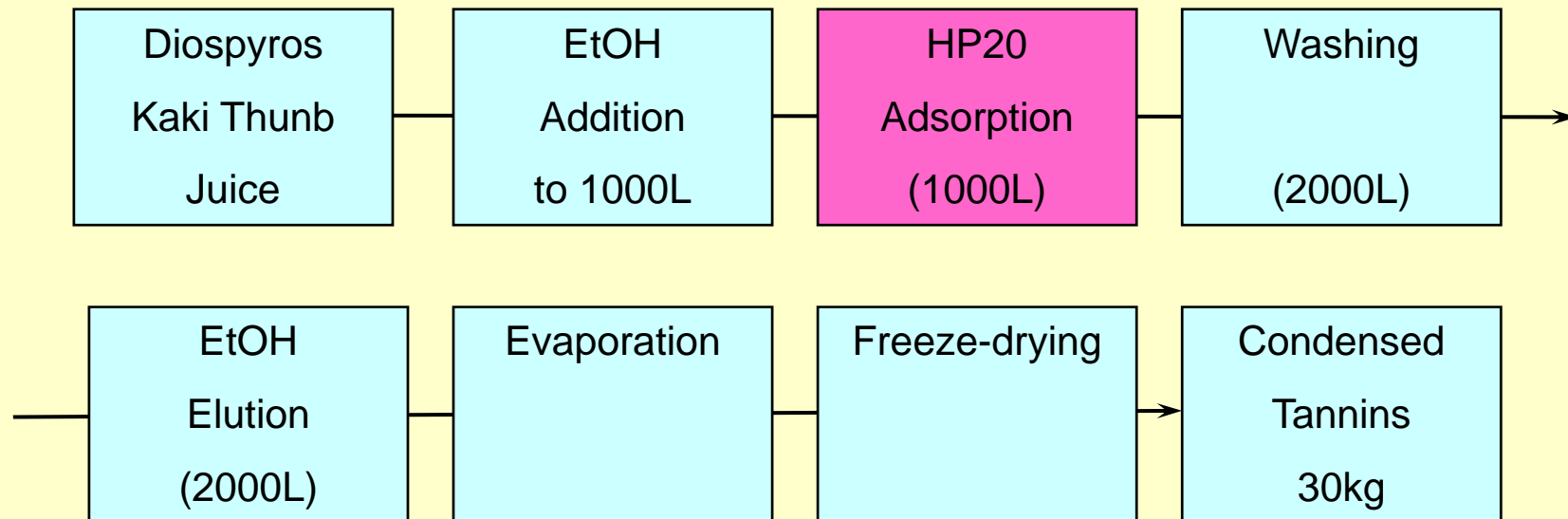
Applications from Patents – Separation of Persimmon Tannins



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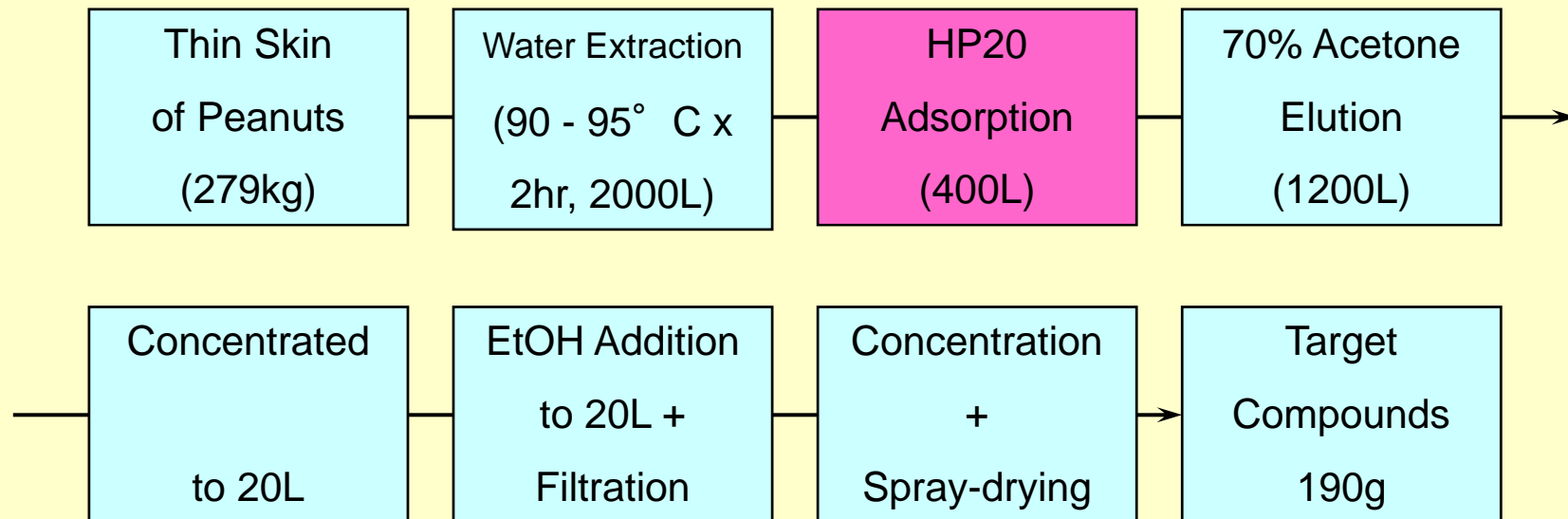
Applications from Patents – Separation of Herbal Drugs

- Mutation inhibitors comprising condensed tannins from Diospyros Kaki Thunb (persimmon)
 - Jpn. Kokai Tokkyo Koho JP 09315985 (Kanebo, Ltd., Japan)



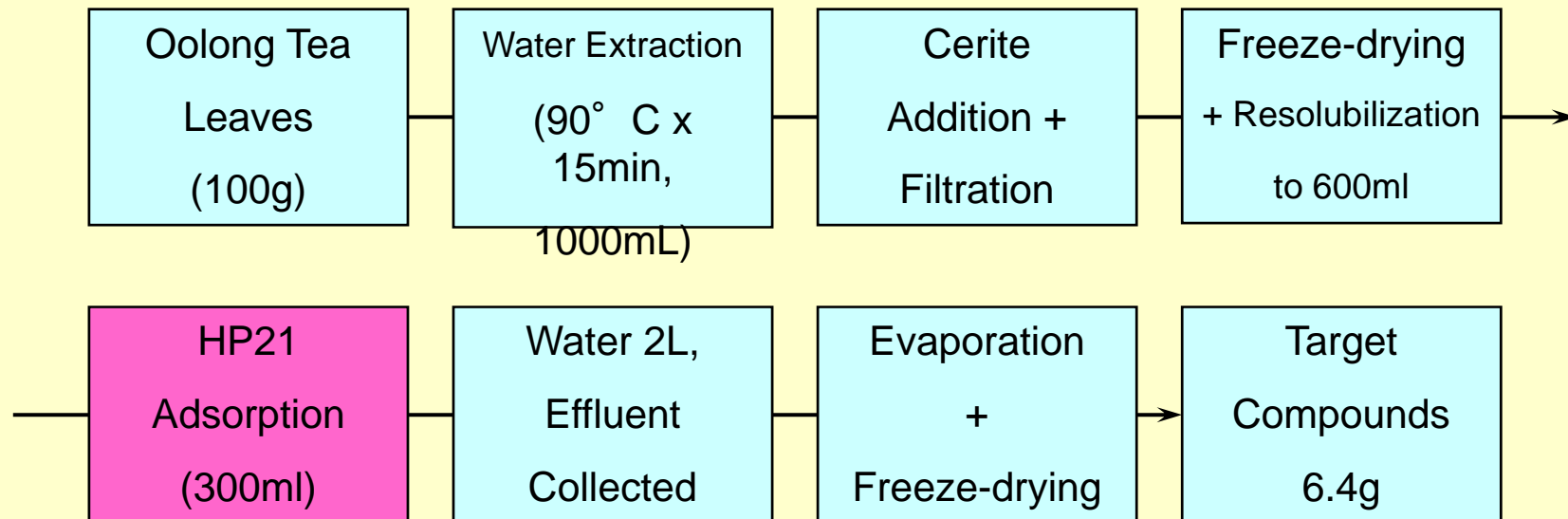
Applications from Patents – Separation of Herbal Drugs

- Anti-HIV agent from *A. hypogaea* (peanut)
 - Jpn. Kokai Tokkyo Koho JP 11246431
(Agency of Industrial Sciences and Technology; Tokiwa Shokubutsu Kagaku Kenkyusho Ltd., Japan)



Applications from Patents – Separation of Herbal Drugs

- Food for tooth decay prevention from Oolong Tea
- Jpn. Kokai Tokkyo Koho JP 04178320 (Suntory, Ltd., Japan)



New Application: Normal Phase Separation with synthetic adsorbents

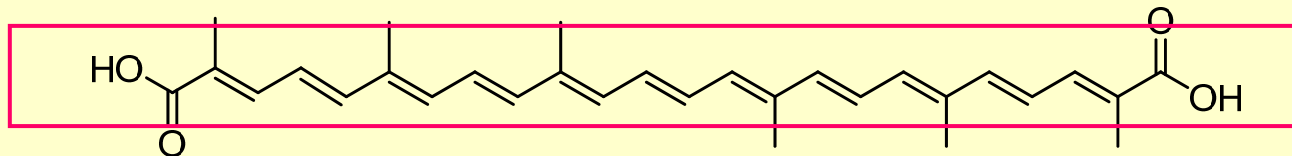
■ Separation of carotenoide of *Bacillus firmus* GB1

- 1st purification by styrene body adsorbent of 100 μ m
- Final purification done with 10 μ m packed column

✘ The separation was difficult with silica gel in normal phase mode or ODS in reversed phase mode.

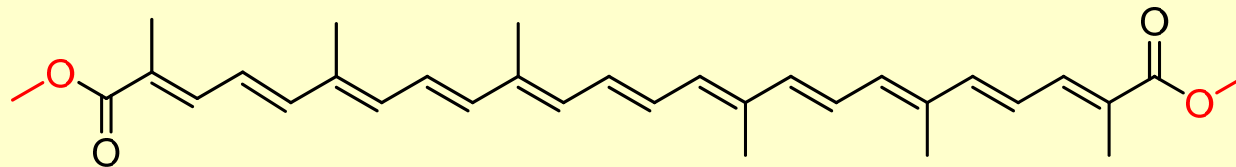
With our styrene body synthetic adsorbent, these difficulties were solved due to its strong interaction by pi-pi interaction.

Natural product

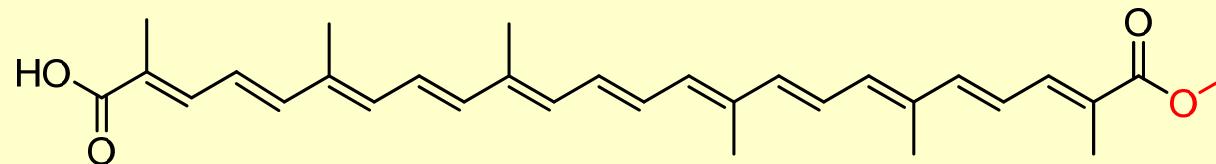


4,4'-diapolycopene dioic acid (1) [1.0 mg]

Methylated derivatives



4,4'-diapolycopene dioic acid dimethylester (2) [2.6 mg]



4,4'-diapolycopene dioic acid monomethylester (3) [0.9 mg]

Data obtained by Professor Shindo and Assistant professor Oosawa of Nippon women university

Isolation scheme for 4,4'-diapolycopene dioic acid (1)

mycelium (from 10 L culture) collected by centrifugation

extracted by MeOH with sonication
partitioned with *n*-hexane – MeOH (1:1)

lower layer (MeOH layer)

partitioned with *t*-buthyl methy ether – MeOH - H₂O (5:1:5)

upper layer (*t*-buthyl methy ether – MeOH layer)

HP20SS column *n*-hexane – EtOH (1:3)

red colored fraction [98.5 mg]

preparative HPLC

①column: Develosil C30-UG-5 (10 × 250 mm)
solvent: *t*-bme – MeOH (1:9) → *t*-bme – MeOH – H₂O (70:26:4)
linear gradient, flow rate: 3 mL/min

②column: **MCI GEL CHP20/C10** (4.6 × 250 mm)
solvent: CH₂Cl₂ 100% → CH₂Cl₂ – MeOH (5:1)
linear gradient, flow rate: 1 mL/min

4,4'-diapolycopene dioic acid (1) [Rt 17.2 min, 1.0 mg]

References

- ***DIAION[®] Manuals I, II***
 - Explanation of Products and Applications
- ***Natural Products Isolation***
(Edited by R. J. P. Cannell, 1998, Humana Press Inc., Totowa, NJ)
(ISBN: 0-89603-362-7)
 - Methods of Extraction and Purification from Natural Products
 - Many Applications using DIAION[®] or SEPABEADS[®]