

臺灣市售中藥材 HPLC 圖譜之鑑定

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摘要

臺灣市售中藥材多仰賴進口，中藥材基原，商品及來源極為混亂，為了解市售中藥材品質及成分含量，本計畫針對常用市售二十種中藥材，利用高效率液體層析儀 (HPLC)，以目前各種藥材常用之指標成分為基礎，針對由全省各地購買之藥材作定量或定性分析，供中藥材品質評價參考，同時供藥政單位訂定中藥材標準參考。

前言

市售中藥方劑，因含多味藥材，成分較為複雜，在方劑成分之定量上，迄今仍有許多問題無法克服，而中藥材品質之良窳，亦影響成品中藥方劑之品質，中藥方劑的品管，必先從單味藥材之品質作一規範，即中藥材標準化必先從單味藥材之品質作一規範，本計畫乃利用高效率液體層析儀，針對常用市售二十種中藥材，每種藥材購自全省北、中、南十個不同定點之中藥店，進行成分分析，以各種藥材目前常用之指標成分為基礎進行分析，探討簡便有效之高效率液相層析檢驗法，定出標準規格，以供建立中藥品管之參考。

材料與方法

一、材料：

(一)儀器裝置：

- 1.減壓濃縮機
- 2.紫外燈
- 3.薄層分析板

4. 高效率液體層析儀

(二) 試藥與試液

1. 溶劑：甲醇、氯仿、甲苯、乙酸乙酯、氰甲烷、苯、丙酮、正丁醇、冰醋酸、磷酸、正己烷、磷酸二氫鉀。
2. 茴香醛－硫酸發色液 (Anisaldehyde- H_2SO_4 Spray reagent)：取 0.5ml 茴香醛，加入 9 毫升酒精，0.5 毫升硫酸，0.1 毫升醋酸混合而成。
3. 硫酸顯色劑：取適量濃硫酸配成 10 % 硫酸水溶液。
4. 氯化鐵顯色劑：取適量氯化鐵配成 10 % 甲醇或乙醇溶液。
5. Dragendorff 試劑：
溶液 A：取 0.85 克次硝酸鉍溶於 10 毫升醋酸及 40 毫升水之混合液中。
溶液 B：取 8 克碘化鉀溶於 20 毫升水中。
臨用前取 A 液 5 毫升，B 液 5 毫升，醋酸 20 毫升與水 100 毫升混合後使用。
6. 標準品：購自 Nacalai Tesque 公司。

(三) 檢體：

- | | |
|-----|--|
| 黃芩 | cutellariae Radix(Scutellaria baicalensis Georgi) |
| 黃連 | Coptidis Rhizoma(Coptis chinensis Wallich) |
| 綿茵陳 | Artemisiae capillaris Herba
(Artemisia capillaris Thunberg) |
| 北茵陳 | Origanum vulgare Herba(Origanum vulgare L.) |
| 地黃 | Rehmanniae Radix et Rhizoma
(Rehmannia glutinosa Liboschitz) |
| 延胡索 | Corydalis Tuber
(Corydalis ambigua Chamisso et Schlechtendal) |
| 葛根 | Puerariae Radix
(Pueraria pseudo-hirsuta Tang et Wang) |
| 蒼朮 | Atractylodis Lanceae Rhizoma
(Atractylodes lancea DC) |
| 吳茱萸 | Evodia Fructus
(Evodia rutaecarpa Hookil et Thomson) |

山梔子	Gardeniae Fructus(<i>Gardenia jasminoids</i> Ellis)
龍膽	Gentianae Scabrae(<i>Gentiane scabra</i> Bunge)
人參	Ginseng Radix(<i>Panax ginseng</i> C.A.meyer)
甘草	Glycyrrhizae Radix (<i>Glycyrrhiza uralensis</i> Fischer et DC)
玄參	Scrophulariae Radix (<i>Scrophularia niagpoensis</i> Heinsley)
厚朴	Magnoliae Cortex (<i>Magnolia officinalis</i> Rehder et Wilson)
芍藥	Paeoniae Albifloree Radix (<i>Paeonia albiflorapallas</i> var <i>trichocarpa</i> Bunge)
牡丹皮	Moutan Radicis Cortex(<i>Paeonia mountan</i> Sims)
黃柏	Phellodendri Cortex (<i>Phellodendron amurense</i> Ruprecht)
柴胡	Bupieuri Radix (<i>Bupleurum falcatum</i> L.)
大黃	Rhei Rhizoma (<i>Rheum officinale</i> Baillon.)

各種藥材購自全省北，中，南各地，共十個中藥店。

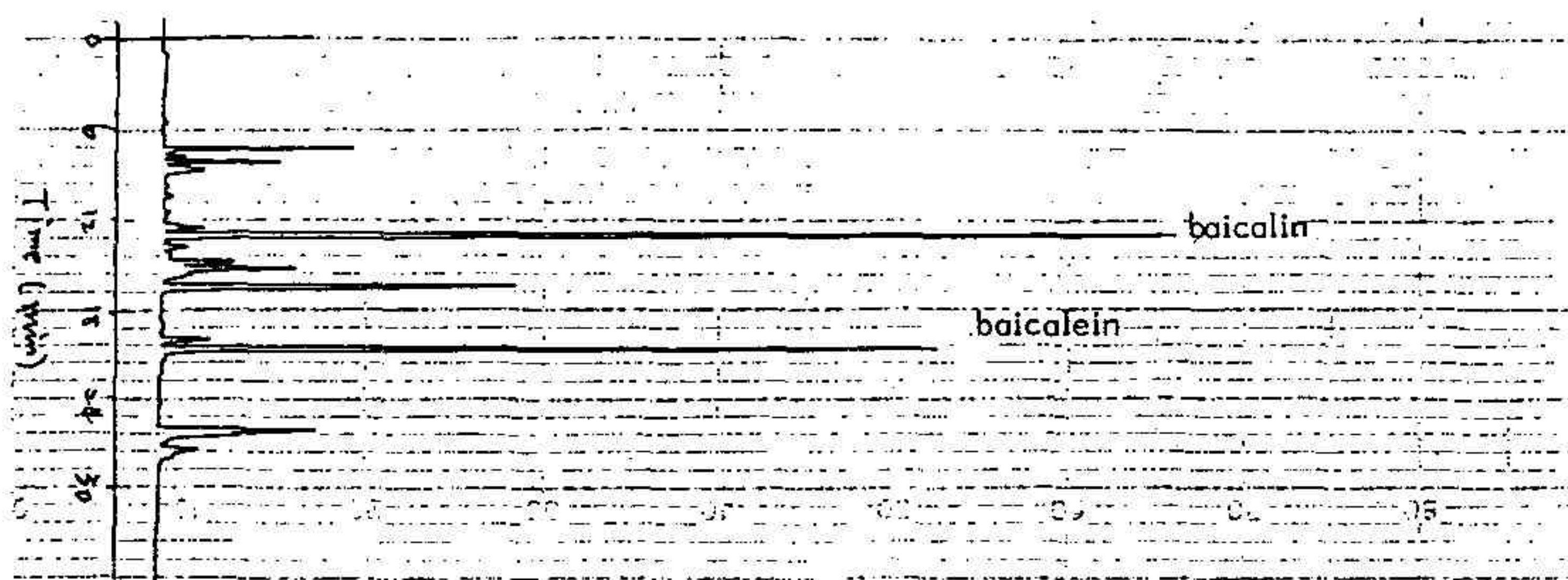
二、方法：

由各中藥店購買之十個不同地點藥材經比對和確認之後，各以 50 % 甲醇／水或甲醇在 Soxhlet。萃取裝置中迴流萃取 4 小時，然後濃縮至一定體積，於經 HPLC 分析前取適量經微過濾器（45 μ ）過濾後，進行 HPLC 及 TLC 分析。

三、結果：

1.HPLC 分析：

1.Scutellariae Radix(Scutellaria baicalensis Georgi)



Alcott 760 HPLC

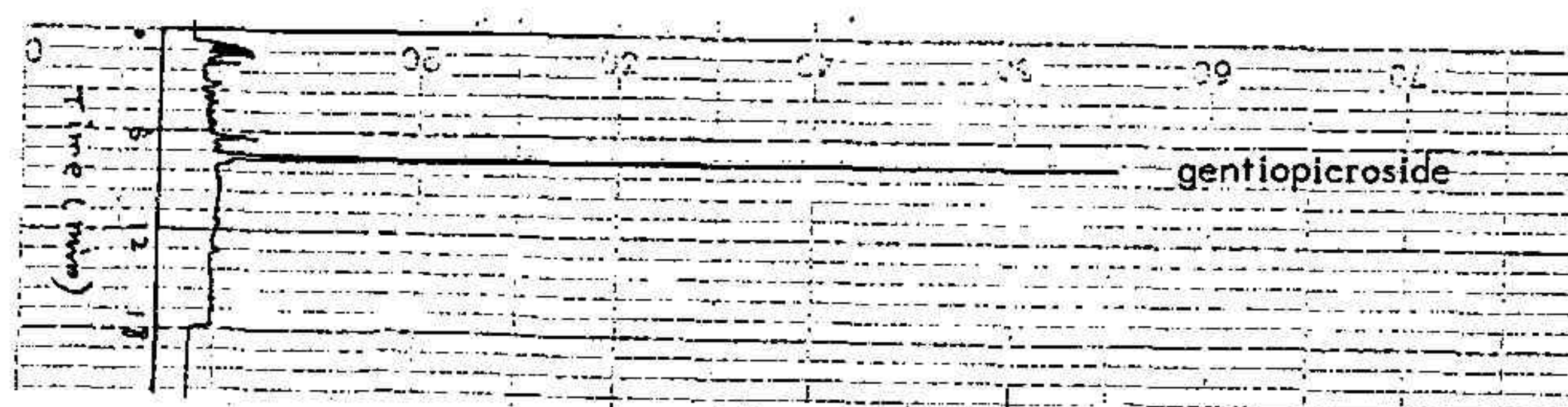
column: Inertsil 4.6x250mm

mobile phase: A: 0.1% H₃PO₄; B: 80% acetonitrile; A/B=80/20→0/100 linear

gradient; flow rate: 1.0ml/min; Detector: 280nm;

sample	1	2	3	4	5	6	7	8	9	10
baicalin(mg/g)	13.44	7.65	11.46	5.43	10.47	6.51	7.76	14.21	13.31	18.68
$y=0.25+1.575x$ $r=0.9996$ $r^2=0.9992$										

2.Gentianae Scabrae(Gentianae scabra Bunge)

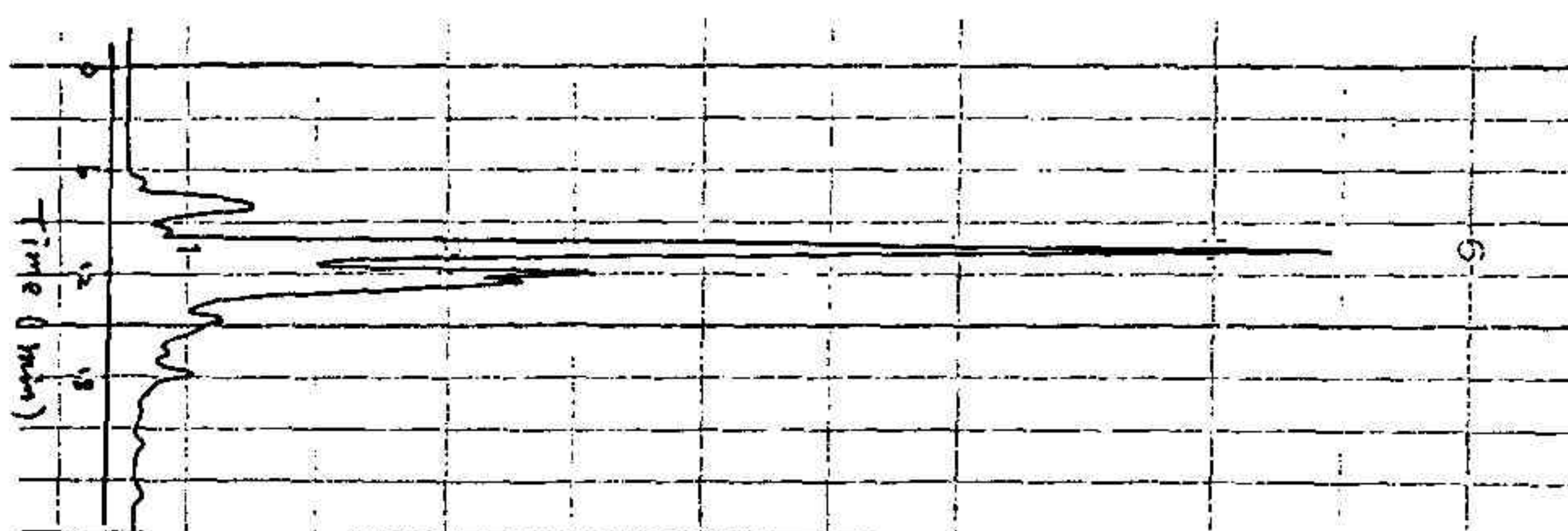


Alcott 760 HPLC

column: VERCOPAK 10 ODS ; mobile phase: A, 20% MeOH in H₂O; b, MeOH; A/B=100/0
--50/50, 10min, 50/50→0/100,22min; Chart speed:1/6 cm/min; Detector:UV 270nm.

sample	1	2	3	4	5	6	7	8	9	10
gentiopicroside (mg/g)	56.92	65.59	61.04	61.63	49.16	71.41	56.78	114.37	45.63	54.07
$y=1.8167+0.45x$ $r=0.9997$ $r^2=0.9993$										

3. *Origanum vulgare* L



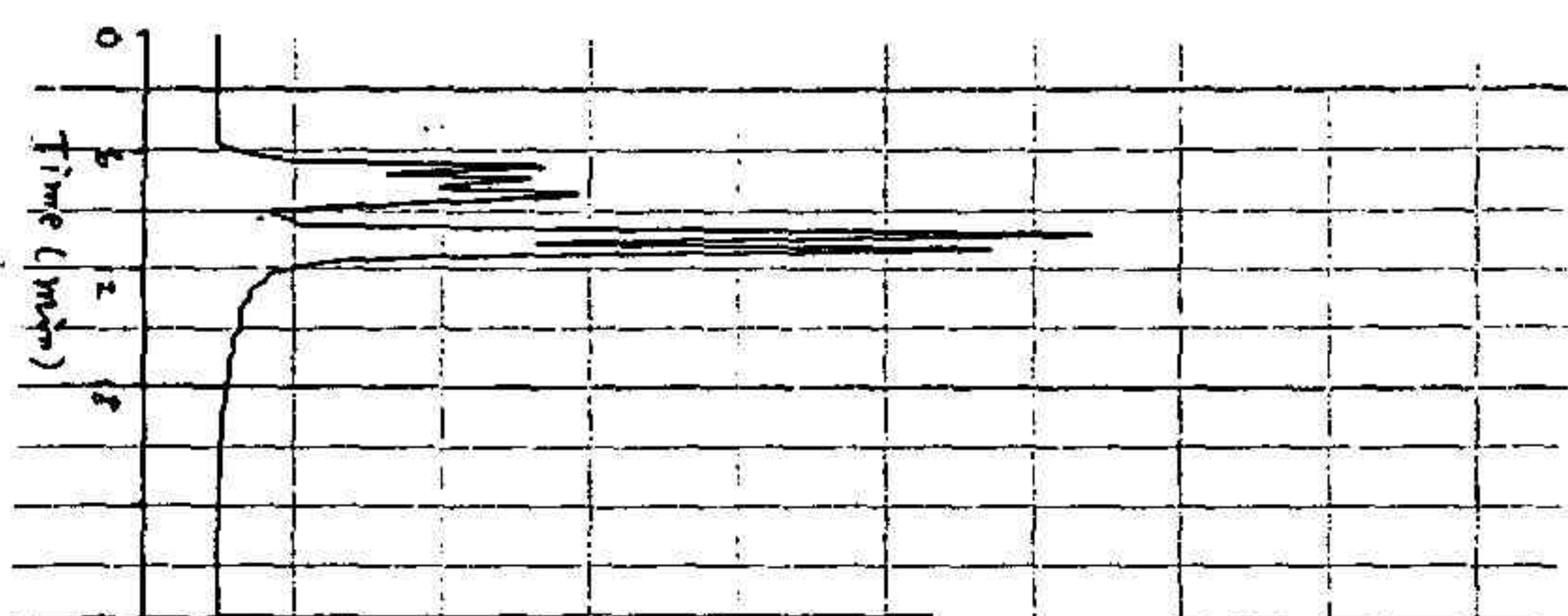
Waters 600E

mobile phase: $\text{H}_2\text{O}:\text{CH}_3\text{CN}:\text{H}_3\text{PO}_4=60:40:0.1\%$

Detector: UV 280nm

Column: VERCOPAK C_{18}

4. *Artemisiae Capillaris* Herb (*Artemisia capillaris*)



waters 600E

mobile phase: $\text{H}_2\text{O}:\text{CH}_3\text{CN}:\text{H}_3\text{PO}_4=60:40:0.1\%$

Detector: UV 280nm

Column: VERCOPAK C_{18}

兩者均不含 capillarsin

5. *Phellodendri* Cortex



Alcott 760 HPLC

Column: Chemcosorb C_{18}

$0.1 \text{ M KH}_2\text{PO}_4:\text{CH}_3\text{CN}=50:50 + \text{SDS } 0.5 \text{ g/l}$; Chart speed: $1/6 \text{ cm/min}$

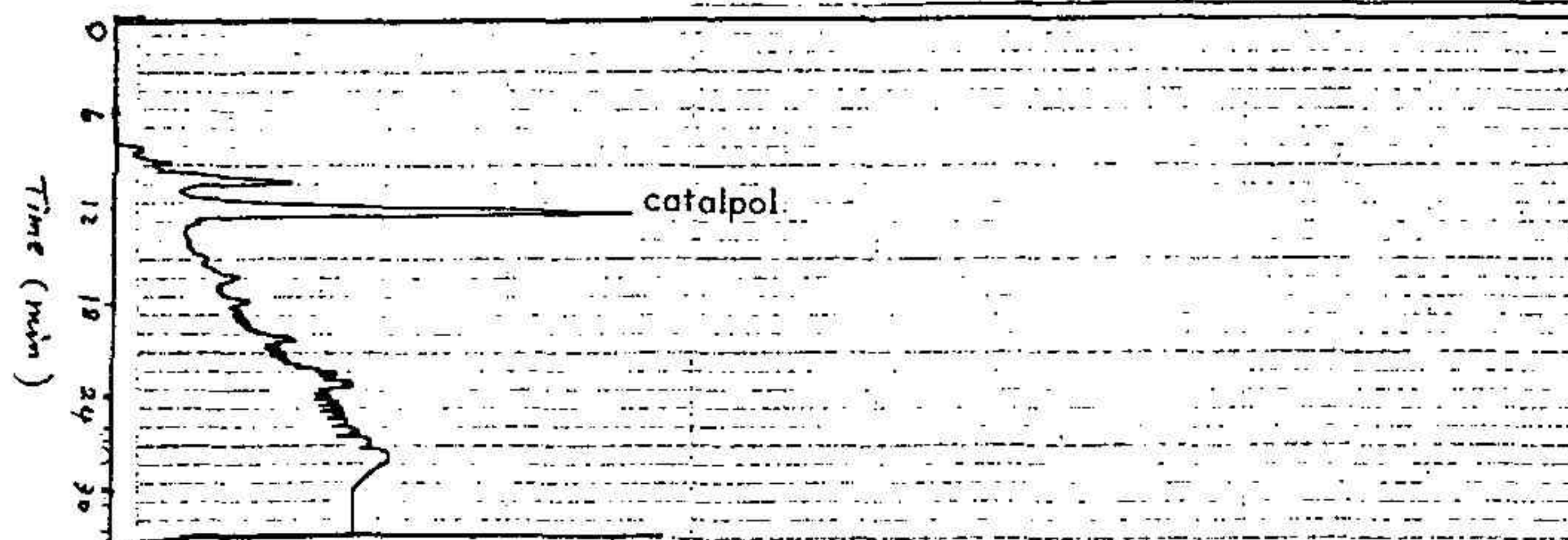
Detector: UV 267nm

$$Y=0.3+2.0786X \quad r=0.99993 \quad r^2=0.99986$$

sample	1	2	3	4	5	6	7	8	9	10
berberine	30.165	7.178	4.234	3.175	36.487	9.410	29.097	4.109	3.310	4.147

(mg/g)

6. *Rehmannia Radix*(*Rehmannia glutinosa* Liboschitz var. *hueichingensis* Chao et Shih)



Alcott 760 HPLC , Detector: low UV 203nm

Column: VERCOPAK C_{18} ; mobile phase: A, H_2O , B, 2% CH_3CN in H_2O ,

A/B= 100/0 $\rightarrow$ 45/55, 10min, 45/55 $\rightarrow$ 0/100, 20min, 0/100 $\rightarrow$ 0/100, 2 min

Chart speed: 1/6 cm/min

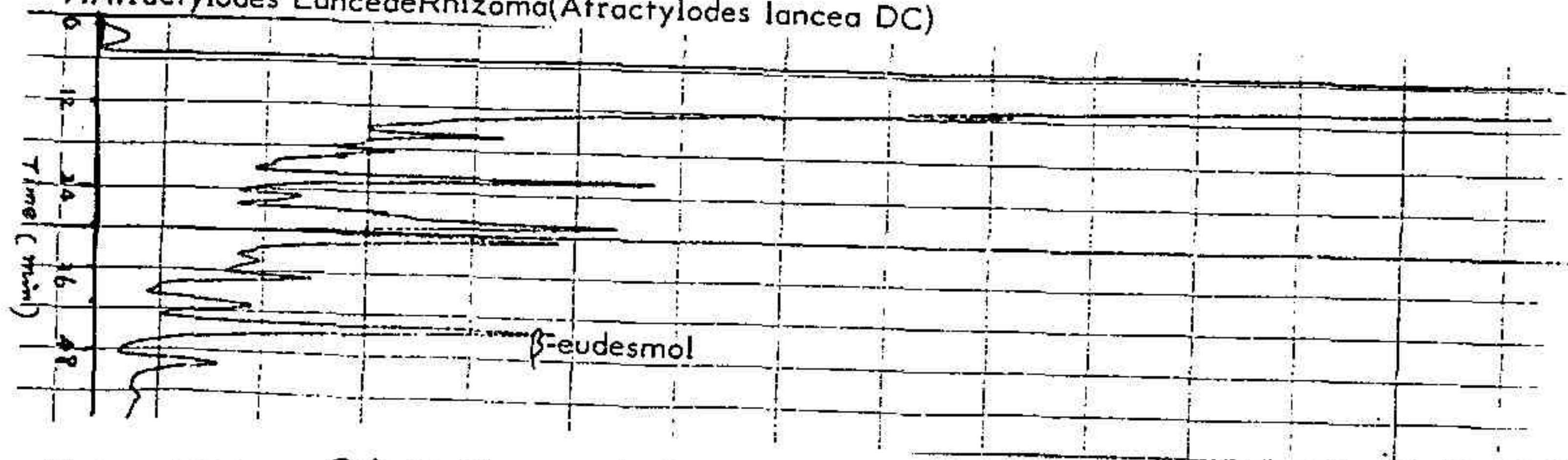
linear gradient,

$$Y=0.0696+1.7893X, \quad r=0.9999, \quad r^2=0.9997$$

Sample	1	2	3	4	5	6	7	8	9	10
catalpol	1.461	2.039	1.954	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

(mg/g)

7. *Atractylodes Lanceae Rhizoma*(*Atractylodes lancea* DC)



Waters 600E , Column Chemcosorb C_{18} ; Detector

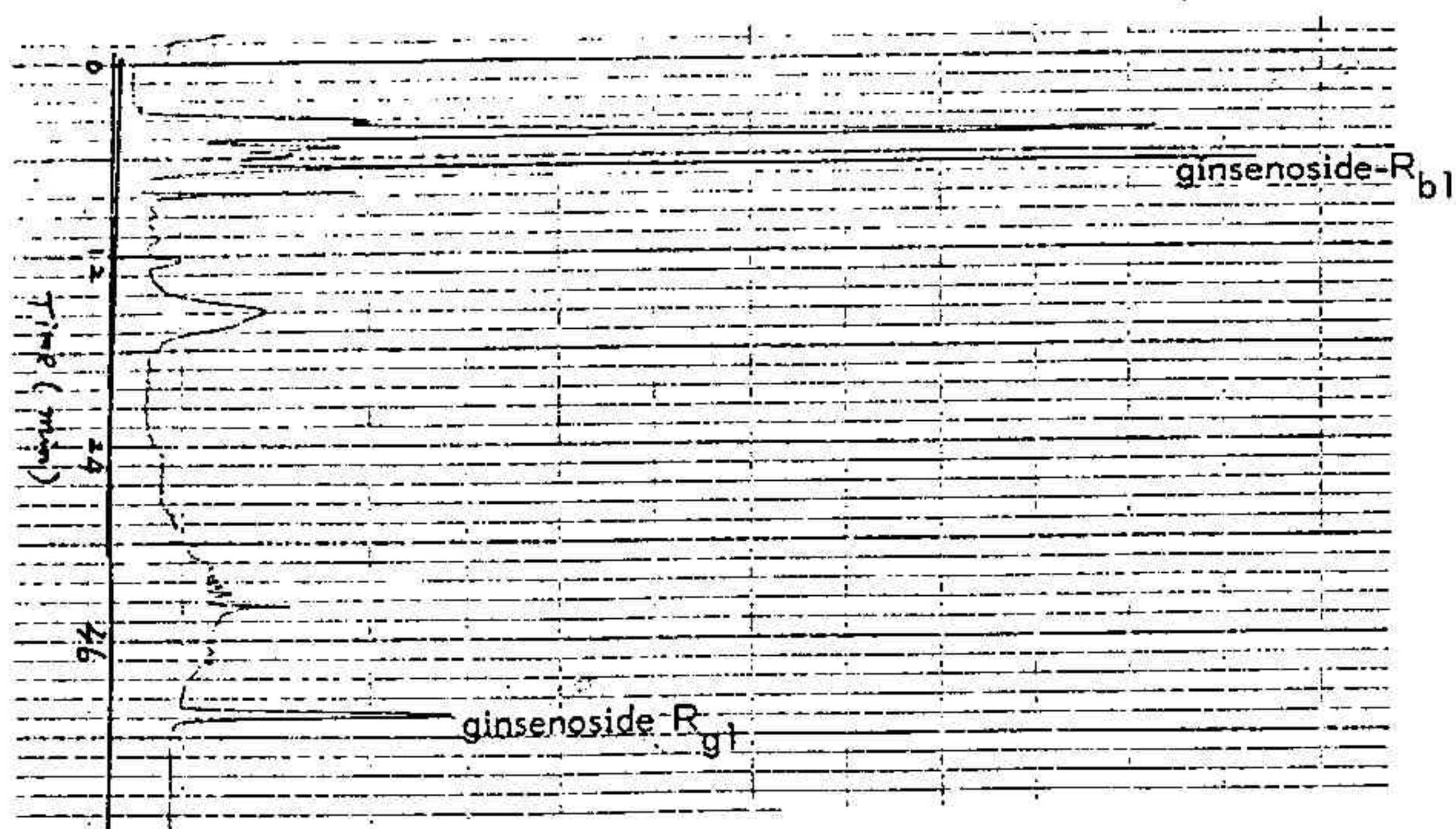
mobile phase: A, $H_2O:CH_3CN:H_3PO_4=95:5:0.1\%$, B, $H_2O:CH_3CN:H_3PO_4=10:90:0.1\%$

A/B: 100/0, 50min, 25/75, 10min, 0/100, 10min; flow rate: 0.5cc/min,

Chart speed: 1/12 cm/min

Detector: low UV 203nm

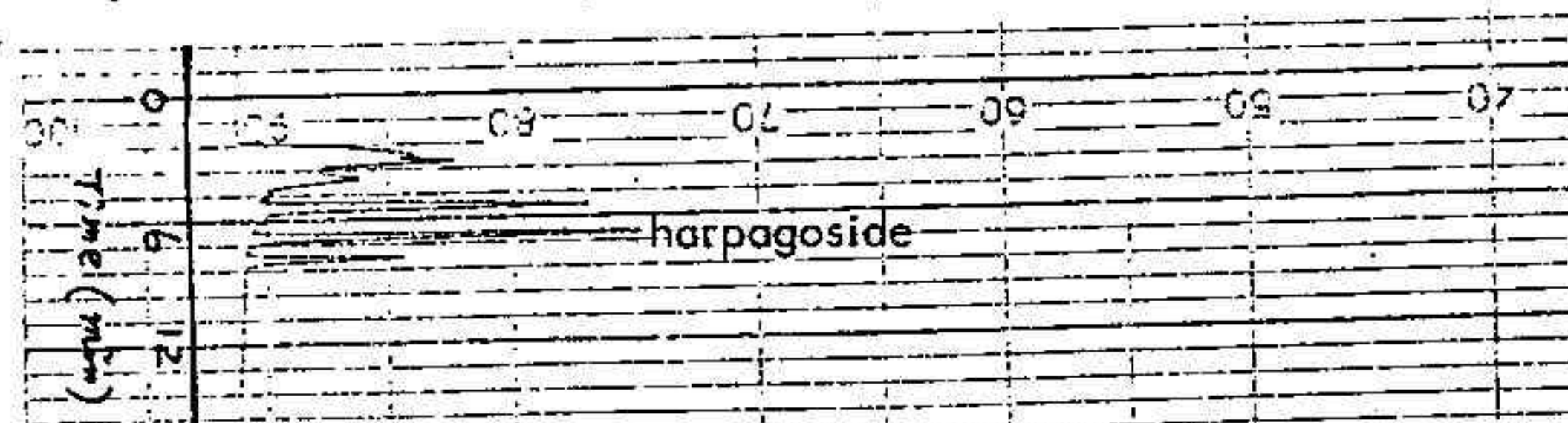
8. Ginseng Radix Rubra (Panax ginseng C.A. Meyer)



Alcott 760 HPLC; Detector: UV 203nm; Column: Chemcosorb C₁₈ 4.6x250mm
 Mobile phase: A, 10% acetonitrile in H₂O, B:30% acetonitrile in H₂O; A/B: 100/0
 20 min → 85/15 → 8 min → 0/100 → 22 min → 0/100

標準品不純品含量。

9. Scrophulariae Radix (Scrophularia niagpoensis Heinsley)

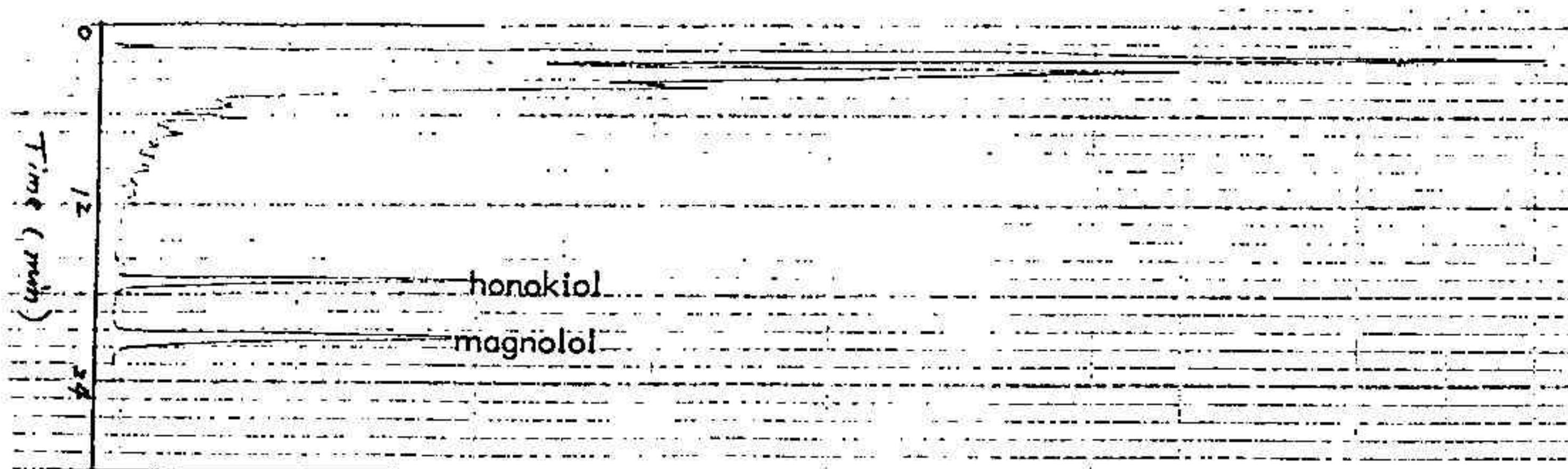


Alcott 760 HPLC ; Column Chemcosorb C₁₈ , 4.6 x250 mm
 Detector: UV 280nm; mobile phase: 30% CH₃CN in H₂O, flow rate: 0.5cc/min
 Chart speed: 1/6 cm/min

$$Y = 1.004 + 4.7786X \quad r = 0.9997 \quad r^2 = 0.9995$$

sample	1	2	3	4	5	6	7	8	9	10
harpagoside	1.716	0.842	1.161	0.659	1.319	1.333	1.815	0.611	0.868	0.744
(mg/g)										

10. Magnoliae Cortex (Magnolia officinalis Rehd. et Wils.)



Alcott 760 HPLC, column: Chemcosorb C₁₈ mobile phase: CH₃CN:H₂O=55:45

Detector: UV 254nm; flow rate: 1cc/min; chart speed: 1/6 cm/min

honokiol: $Y = -0.0194 + 2.3224X$ $r = 0.99997$ $r^2 = 0.99994$

sample	1	2	3	4	5	6	7	8	9	10
honokiol (mg/g)	1.209	0.759	0.437	0.569	0.180	1.065	0.348	0.294	1.418	0.892
magnolol (mg/g)	0.699	0.632	1.835	3.745	2.556	11.168	2.618	0.275	3.924	9.039

magnolol: $Y = 0.2937 + 1.5841X$ $r = 0.9996$ $r^2 = 0.9993$

11. Puerariae Radix (Pueraria pseudo-hirsuta Tang et Wang)



Alcott 760 HPLC Column: Chemcosorb C₁₈

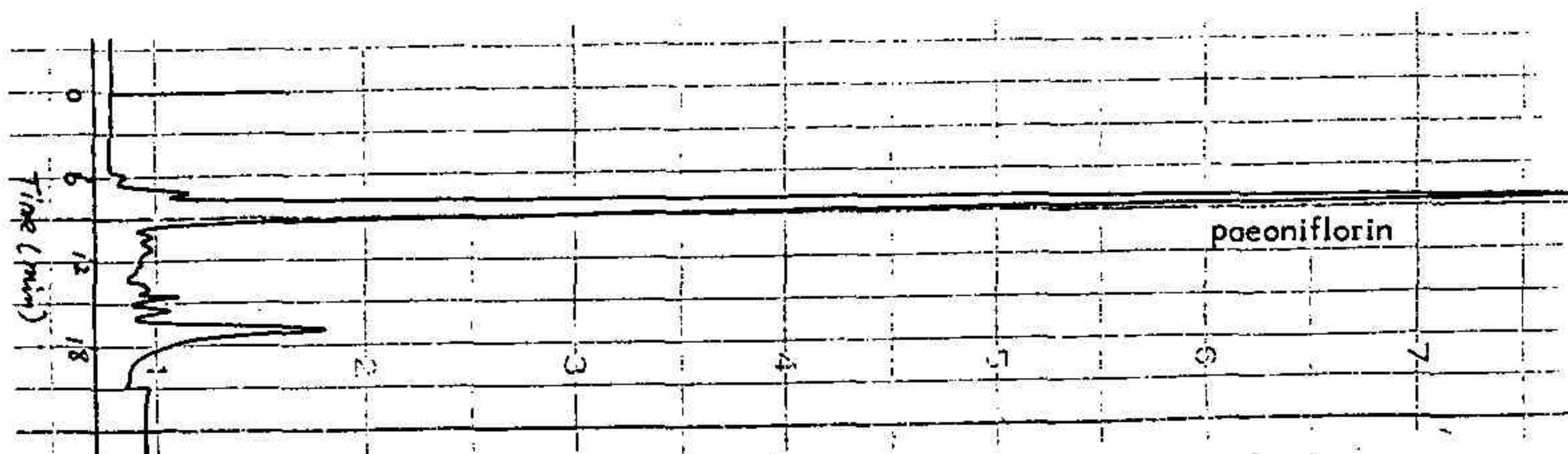
Detector: UV 249nm

Mobile phase: A: 0.05% H₃PO₄ in H₂O, B: CH₃CN: MeOH=40:60

A/B=40/60 → 30/70 → 0/100
20 min 10 min

標準品 daizein 和 puerarin 都很不純, 無法辨認及定量

12. Paeoniae Radix (Paeonia lactiflora Pallas)

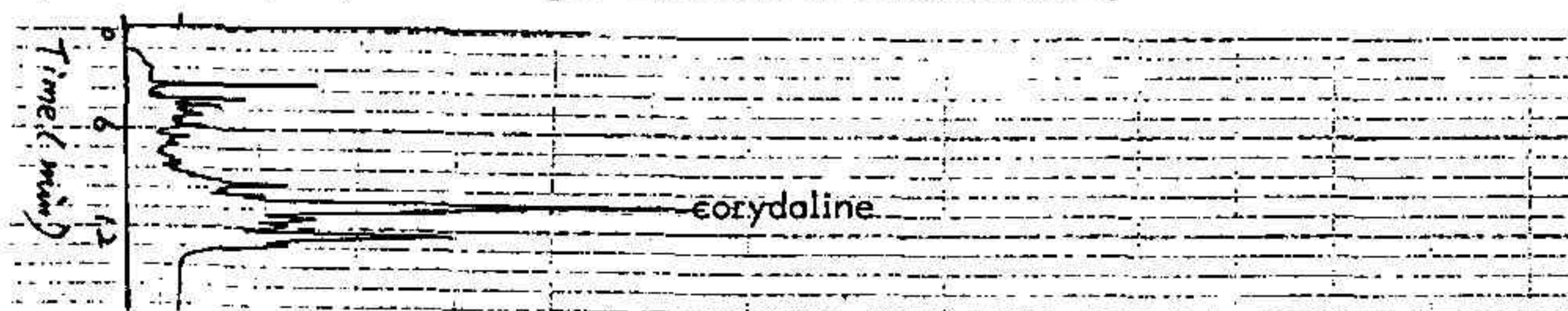


Waters 600E column: Chemcosorb C₁₈ mobile phase: CH₃CN: H₂O=40:60
 Detector: UV 229nm flow rate: 0.5cc/min chart speed: 1/6 cm/min

$$Y = 0.6429 + 4.1929X \quad r = 0.99996 \quad r^2 = 0.99992$$

sample	1	2	3	4	5	6	7	8	9	10
paeoniflorin (mg/g)	9.332	37.810	26.349	30.622	31.556	39.307	9.415	40.957	21.539	18.140

13. Corydalis Tuber (Corydalis ambigua Chamisso et Schlechtendal)



Alcott 760 HPLC Column: Chemcosorb C₁₈ Detector: UV 280nm

Mobile phase: A: CH₃CN: H₂O: H₃PO₄: C₆H₁₅N = 5:95:0.1%:0.01% (0.1ml/l),

B: CH₃CN: H₂O: H₃PO₄: C₆H₁₅N = 90: 10: 0.1%: 0.01%

A/B: 100/0 → 60/40 → 0/100 → 0/100
 40 min 15 min 10 min

Flow rate: 0.5cc/min

Chart speed: 1/6 cm/min

標準品 corydaline 雜有相當量雜質未加含量:

14. Avodia Fructus (Evodia rutaecarpa)



Alcott 760 HPLC

Column: Chemcosorb C₁₈ 4.6x 250mm

Detector: UV 254 nm

Mobile phase: A: 5%CH₃CN in H₂O:0.1% H₃PO₄ + C₆H₁₅N(0.1cc/l)

B: CH₃CN: H₂O: H₃PO₄: C₆H₁₅N =90: 10 :0.1% :0.01%(0.1 ml/l)

A/B: 100/0 → 60/40 → 0/100 → 0/100
40 min 15 min 10 min

Flow rate: 0.5 ml/min

Chart speed: 1/6 cm/min

15.Coptidis Rhizoma



Alcott 760 HPLC

Column: VERCOPAK C₁₈

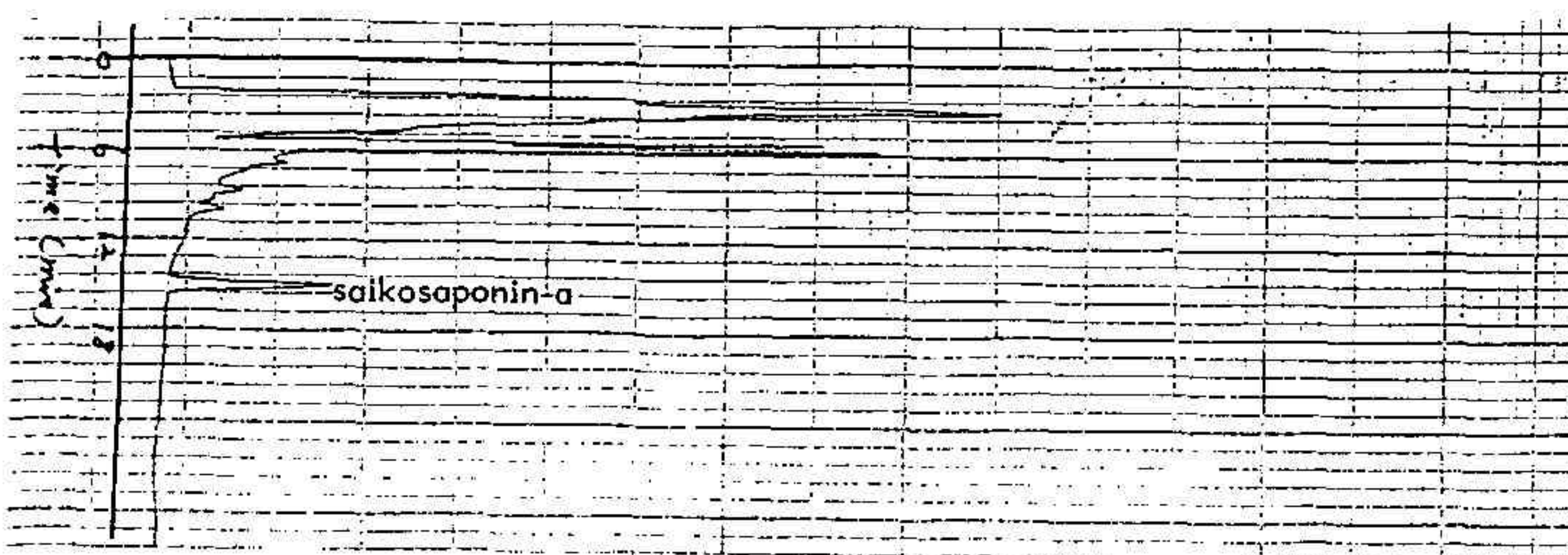
Detector: UV 267 nm

Mobile phase: 0.1M H₃PO₄: CH₃CN=50: 50 + SDS 0.5 g/l

Flow rate : 0.5 ml/min

Chart Speed: 1/6 cm/min

16.Bupleuri Radix



16.

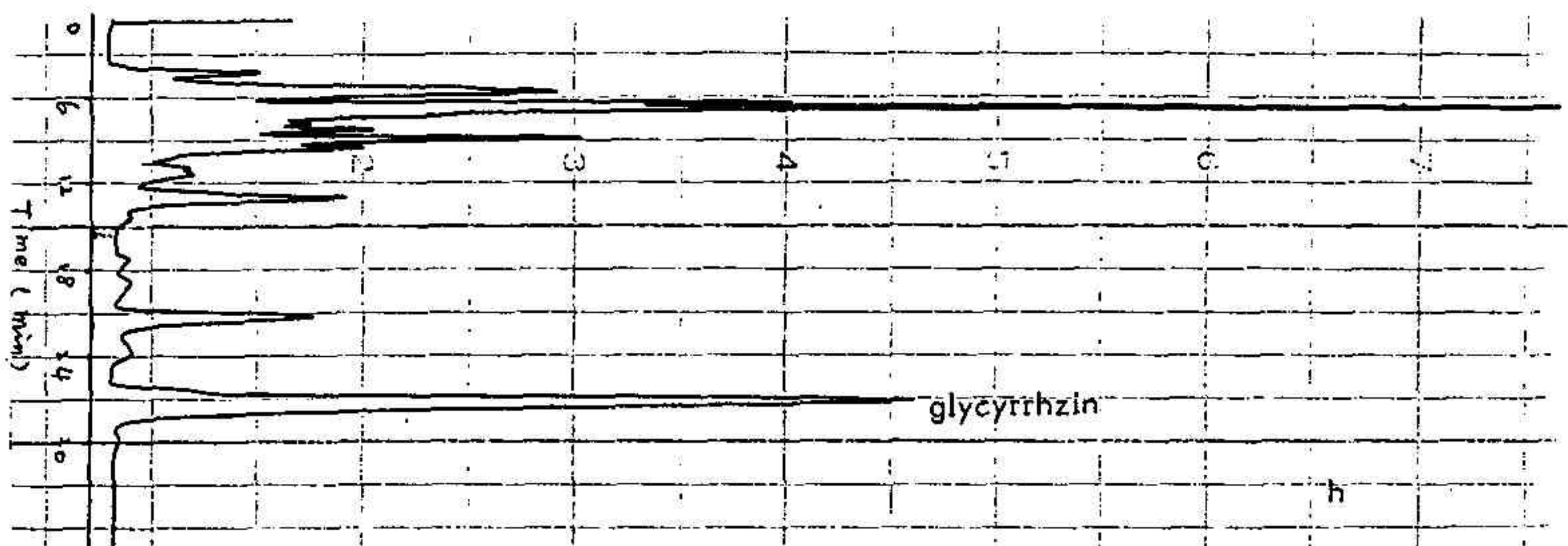
Alcott 760 HPLC

Column: Chemcosorb C₁₈

Mobile phase: A: 10% Acetonitrile, B: 39% acetonitrile

A → B
linear gradient

17. Glycyrrhizae Radix



Waters 600E

Detector: UV 254 nm

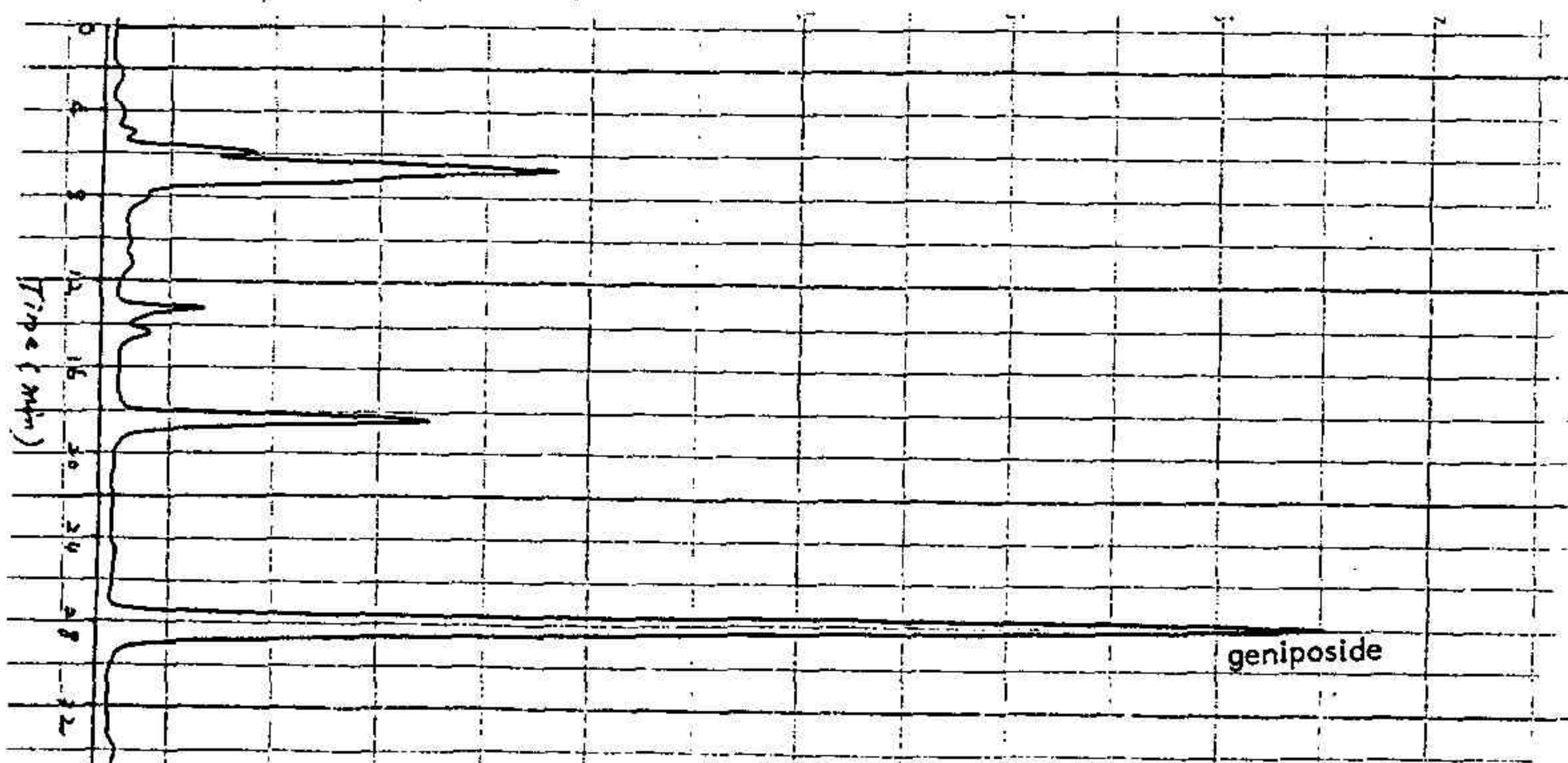
Column: VERCOPAK 4.6 x250 mm

Mobile phase: CH₃CN: H₂O: acetic acid= 40: 60: 1%

flow rate: 0.5cc/min

Chart speed: 1/6 cm/min

18. Gardeniae Fructus(Gardenia jasminoids Ellis)



Waters 600E

Column: Inertsil 5 ODS 4.6 x250 mm

Detector : UV 238 nm

mobile phase: A, H₂O; B, CH₃CN; A/B: 100/0 → 10min → 70/30

Flow rate: 0.6 ml/min

Chart speed: 1/4 cm/min

20 min → 0/100 → 2 min → 0/100

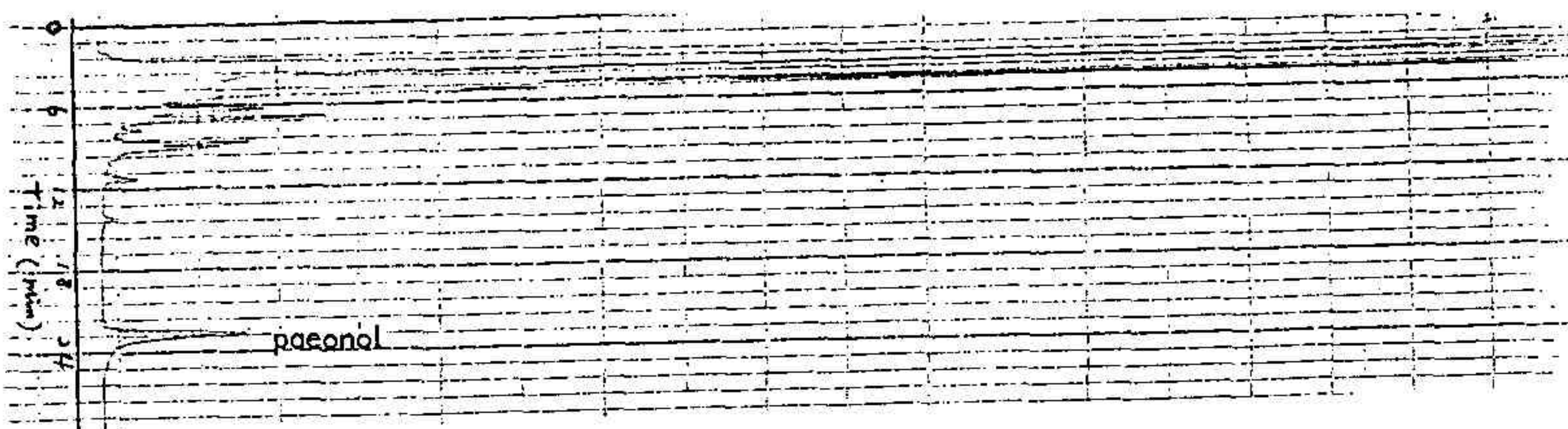
$$18. Y = 0.2211 + 0.6132X$$

$$r = 0.9990$$

$$r^2 = 0.9980$$

sample	1	2	3	4	5	6	7	8	9	10
geniposide	45.949	30.918	51.557	77.718	71.086	44.630	63.833	79.167	50.571	67.21
(mg/g)										

19. Moutan Radicis Cortex (Paeonia moutan Sims)



Alcott 760 HPLC

Flow rate: 0.6 ml/min

Column: Nucleosil C₁₈

$$Y = -5215.56 + 13998.04 X$$

Detector: UV 273 nm

Chart speed: 1/6 cm/min

$$r = 0.9998$$

Mobile phase: CH₃CN : H₂O : H₃PO₄

= 40: 60: 0.1%

$$r^2 = 0.9997$$

sample	1	2	3	4	5	6	7	8	9	10
paeonol	27.797	5.839	1.235	7.810	6.150	2.849	4.157	2.789	1.979	3.959
(mg/g)										

20. Rhei Rhizoma



Alcott 760 HPLC

Detector: UV 254 nm

Column: Chemcosorb C₁₈, 4.6 x 250 mm

Mobile phase: CH₃CN : H₂O : H₃PO₄ = 75: 25: 0.1%

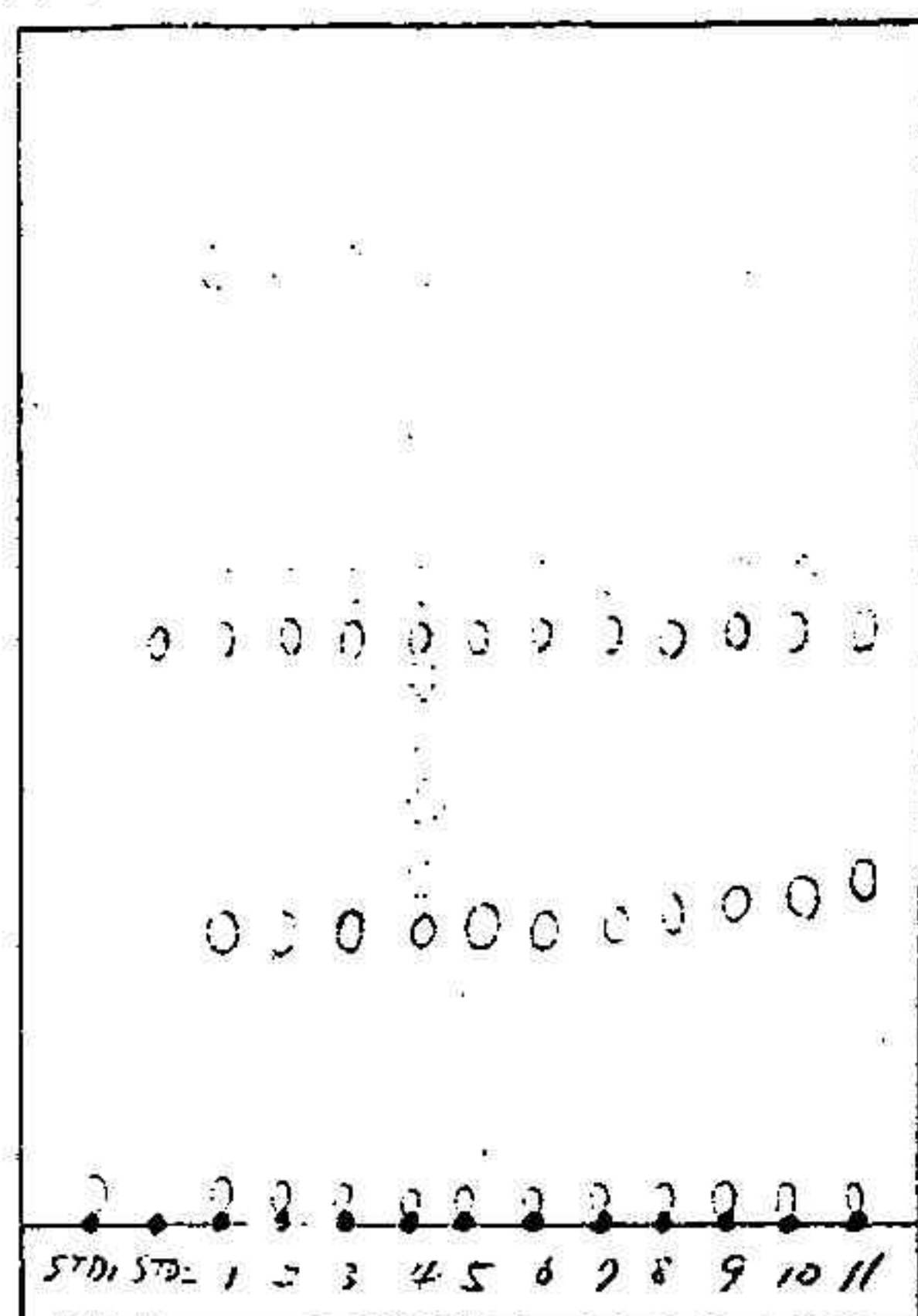
Flow rate: 0.6 ml/min

Chart speed: 1/6 cm/min

標準品都不能定量

2. TLC 分析:
黄芩

Scutellariae Radix

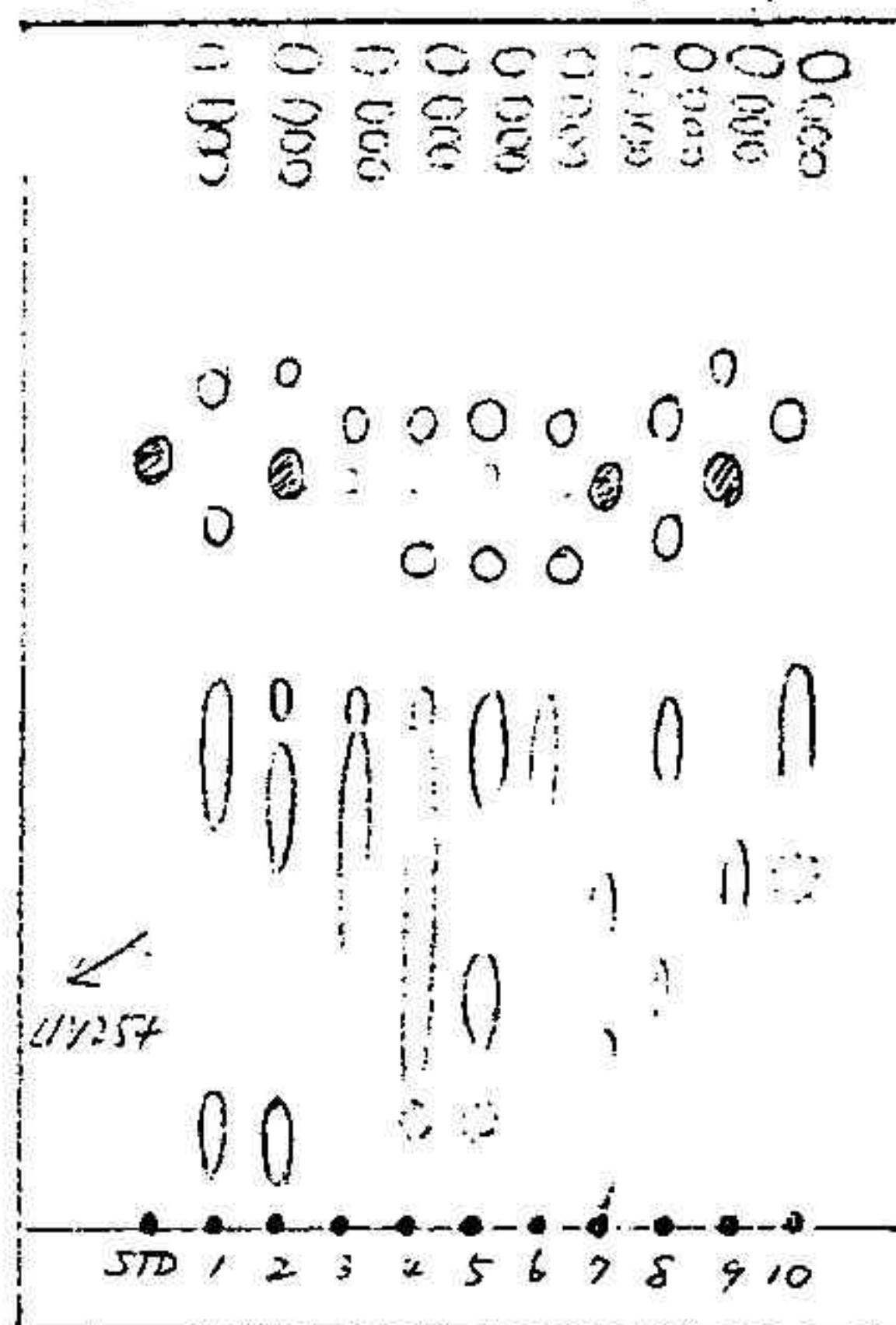


STD₁: baicalein, STD₂: wogonin

solvent system: Toluene:ethyl acetate:MeOH
=8:1:1

sprayer: 1% FeCl₃-MeOH

地黄 Rehmanniae Radix et Rhizoma

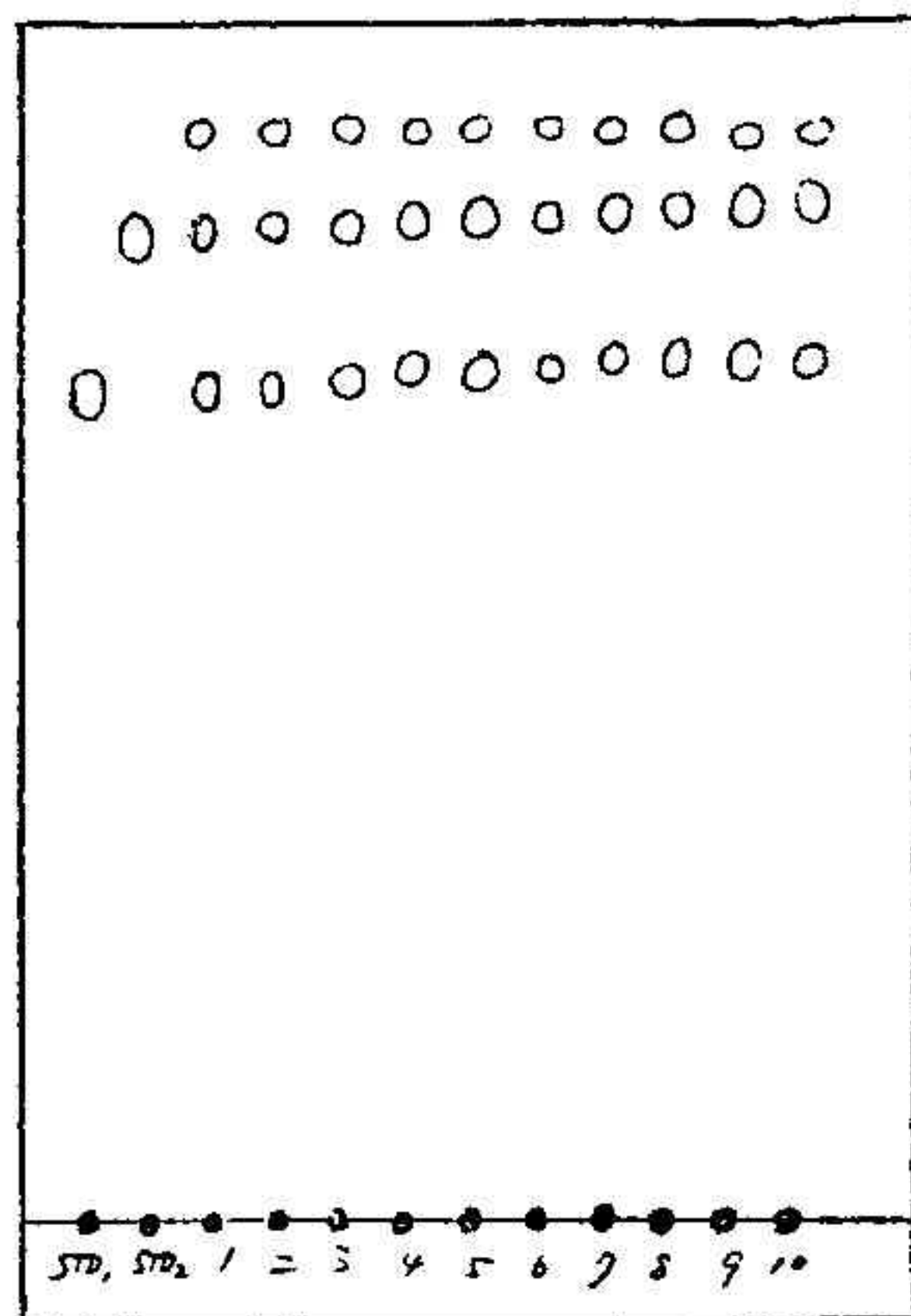


STD: catalpol

solvent system: CHCl₃:MeOH:H₂O=6:4:1

sprayer: p-anisaldehyde/H₃PO₄

延胡索 Corydalis Tuber



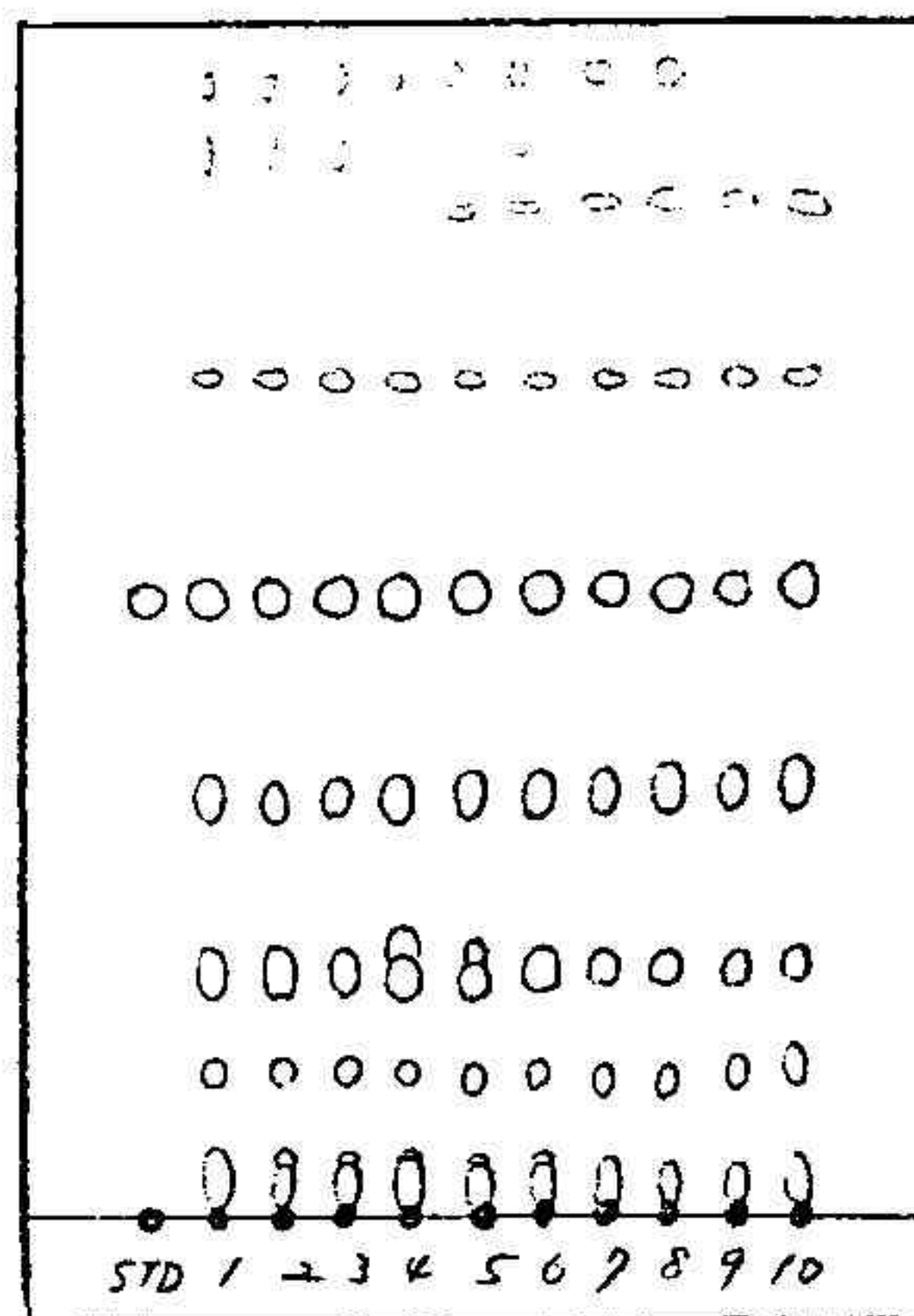
STD₁: corydaline

STD₂: tetrahydro-
palmatine

Solvent system: CHCl₃: MeOH=97.5%:2.5%

Sprayer: Dragendorff

山梔子 Gardeniae Fructus

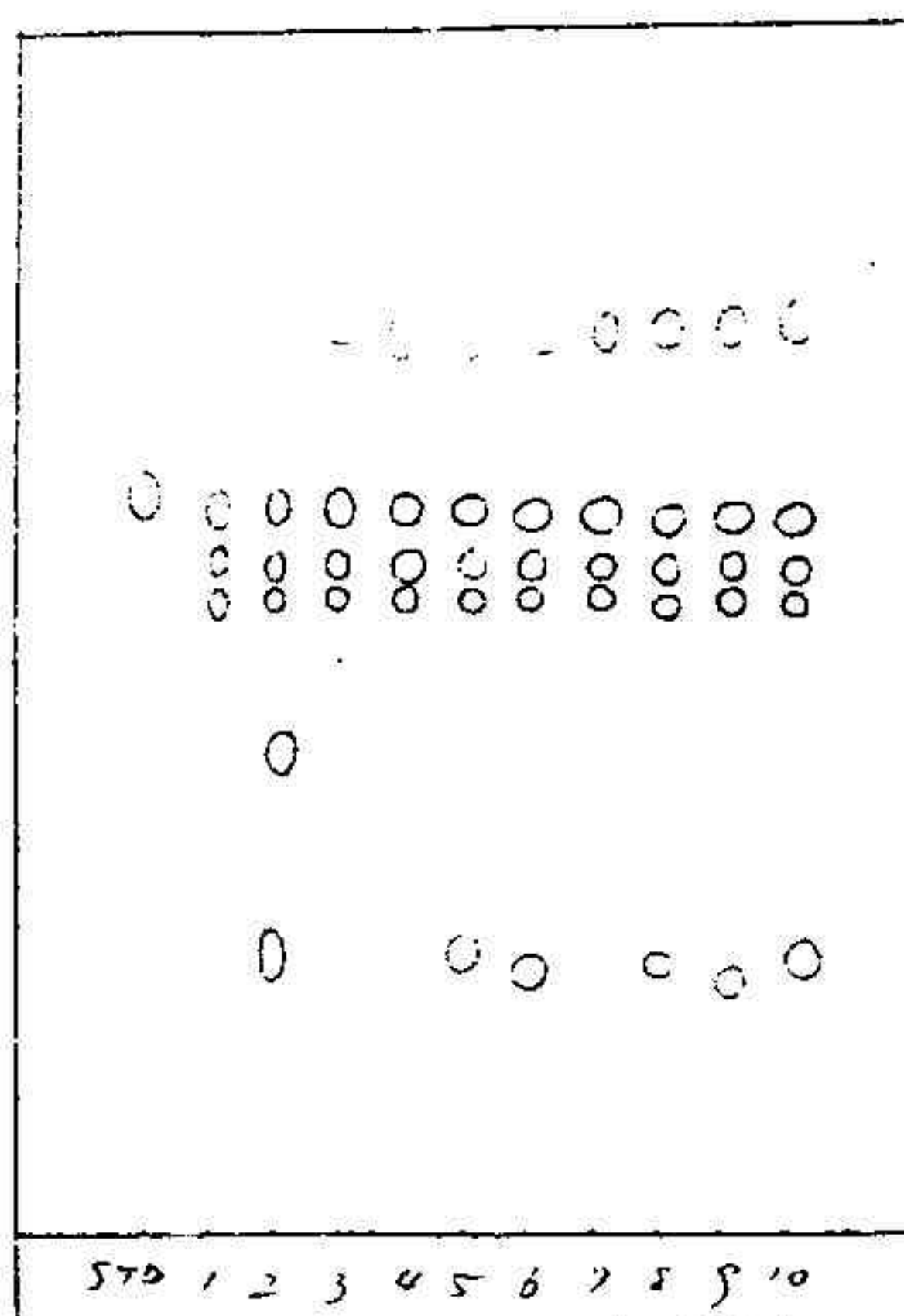


STD: geniposide

Solvent system: p-anisaldehyde/ H₂SO₄

Sprayer: p-anisaldehyde/ H₂SO₄

黃連 *Coptidis Rhizoma*

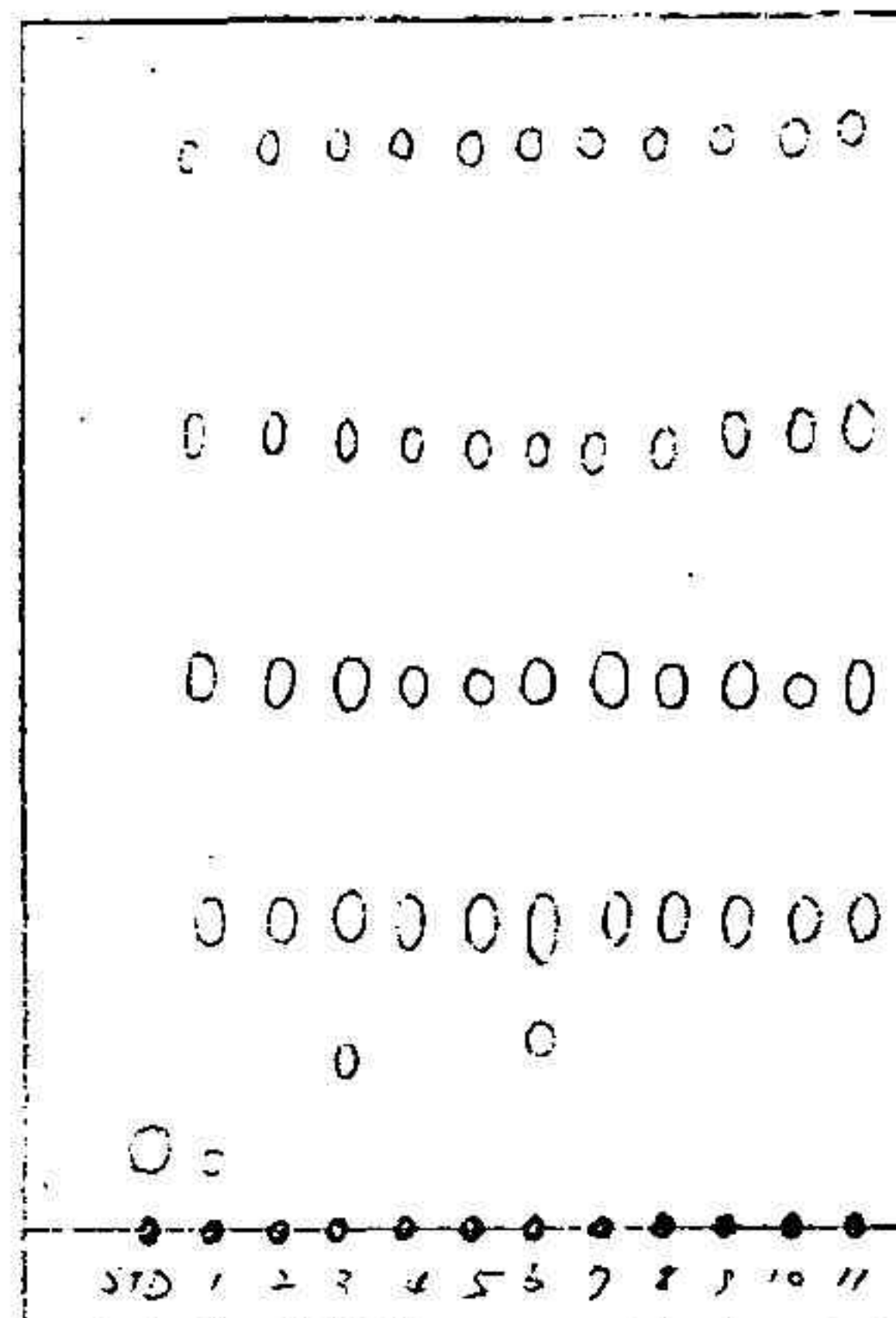


STD: berberine

Solvent system: n-butanol: acetic acid
: H₂O = 7:2:1

Detection: UV, Dragendorff

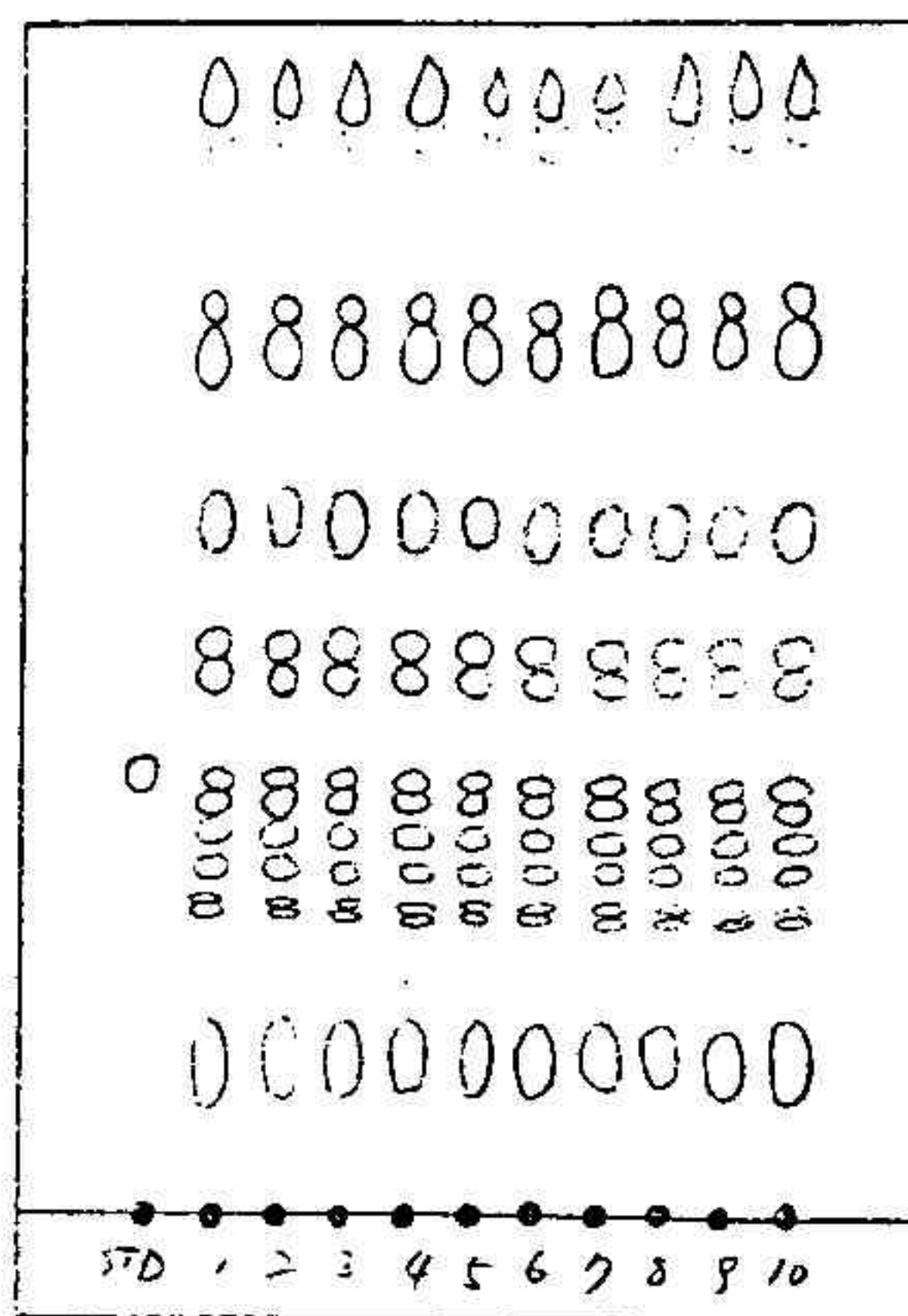
綿茵陳: *Artemisiae Capillaris Herba*



STD: capillarin

Solvent system: n-hexane: CHCl₃: EA = 4:1:1
Detection: UV, 10% H₂SO₄ Soln

甘草 *Glycyrrhizae Radix*

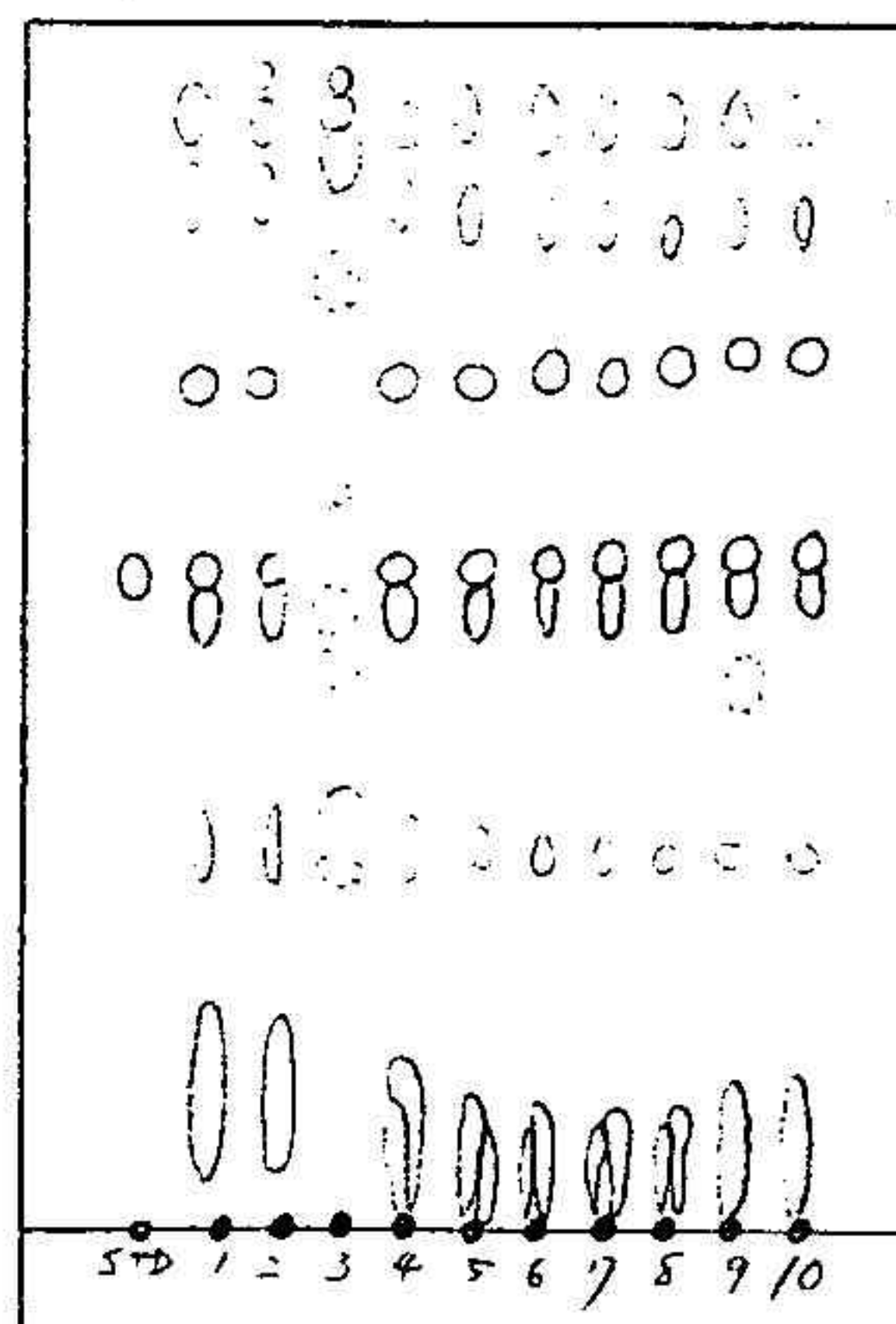


STD: glycyrrhizin

Solvent system: n-butanol: acetic acid: H₂O
= 7: 1: 2

Sprayer: 10% H₂SO₄ soln

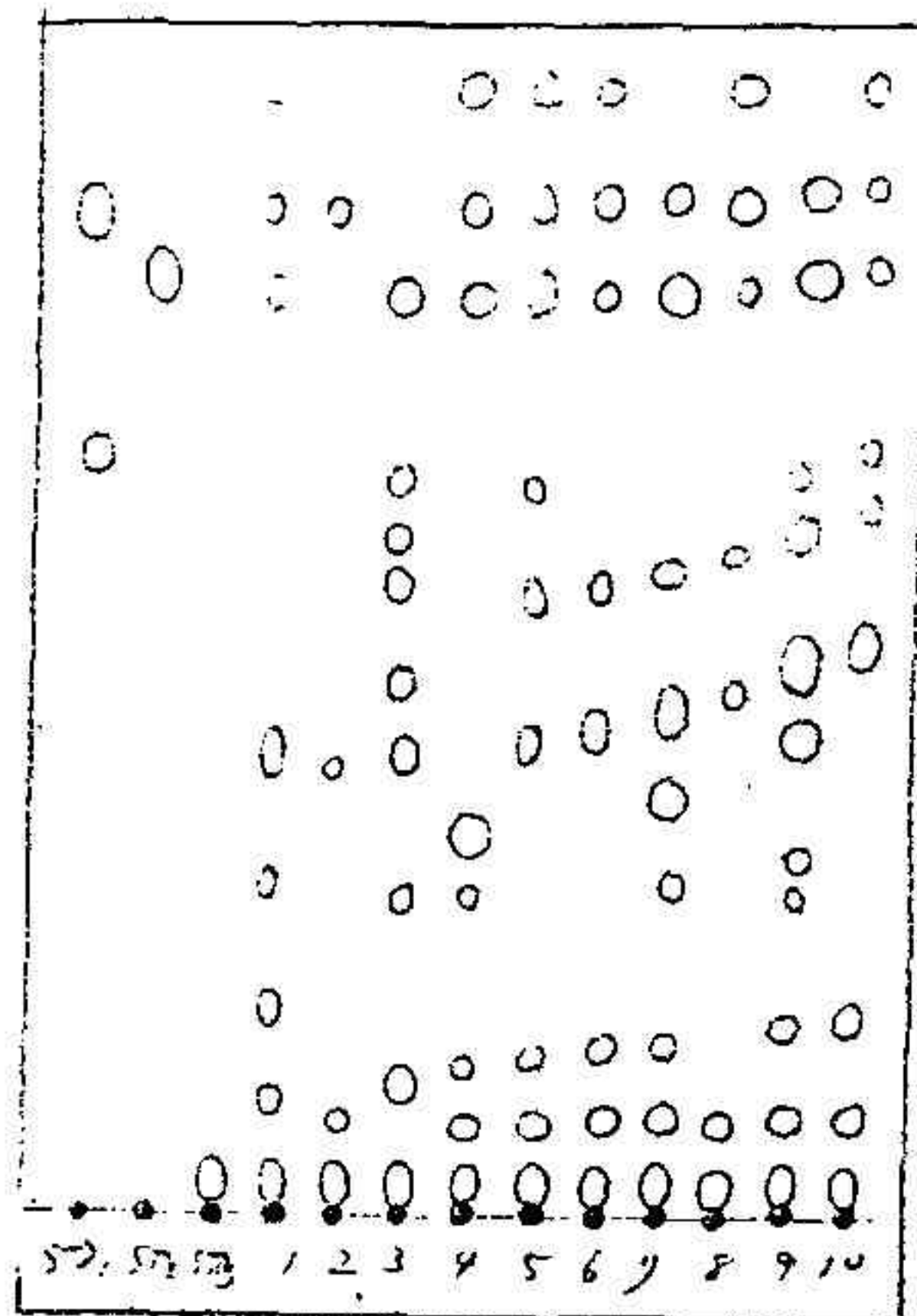
龍膽 *Gentianae Scabrae*



STD: gentiopicroside

Solvent system: n-butanol: acetic acid: H₂O
Sprayer: p-anisaldehyde/H₂SO₄

吴茱萸 *Evodia Fructus*



STD₁: dehydroevodiamine

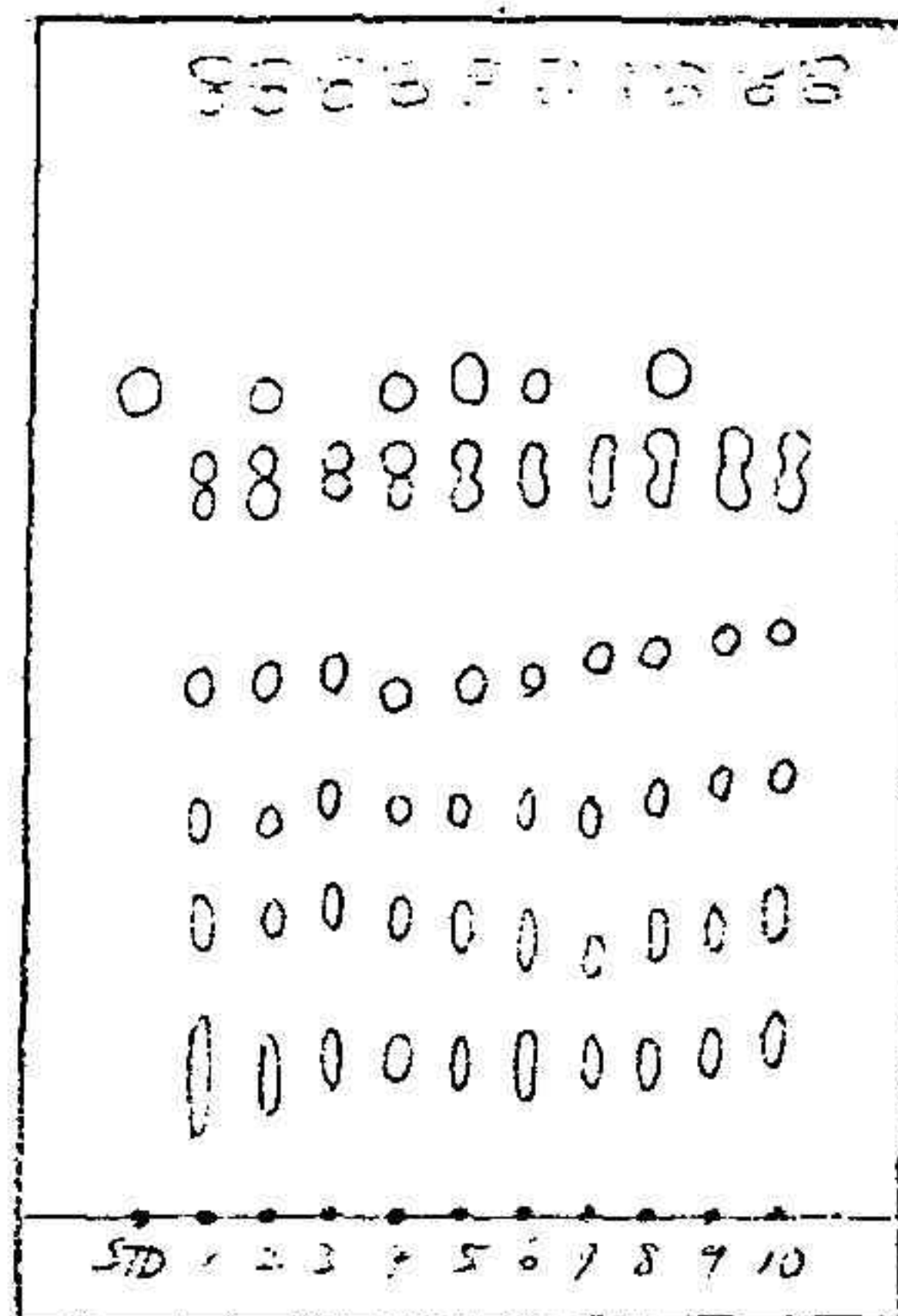
STD₂: evodiamine

STD₃: rutaecarpine

Solvent system: CHCl₃:MeOH=97.5: 2.5

Sprayer: Dragendorff

牡丹皮 *Moutan Radicis Cortex*

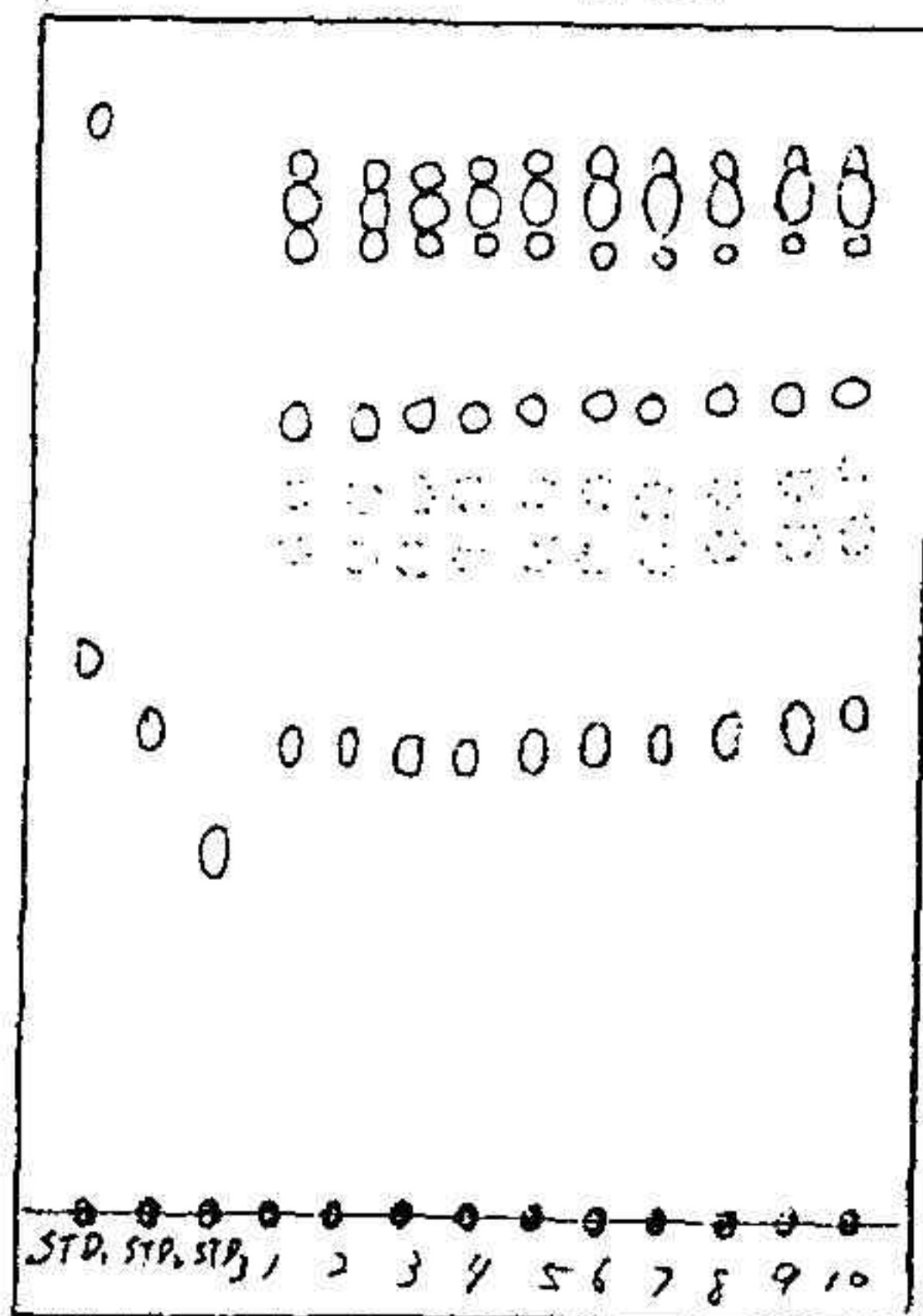


STD: Paeonol

Solvent system: n-hexane: EA=4: 1

Sprayer: p-anisaldehyde/ H₂SO₄

柴胡 *Bupleuri Radix*



STD₁: Saikosaponin-d

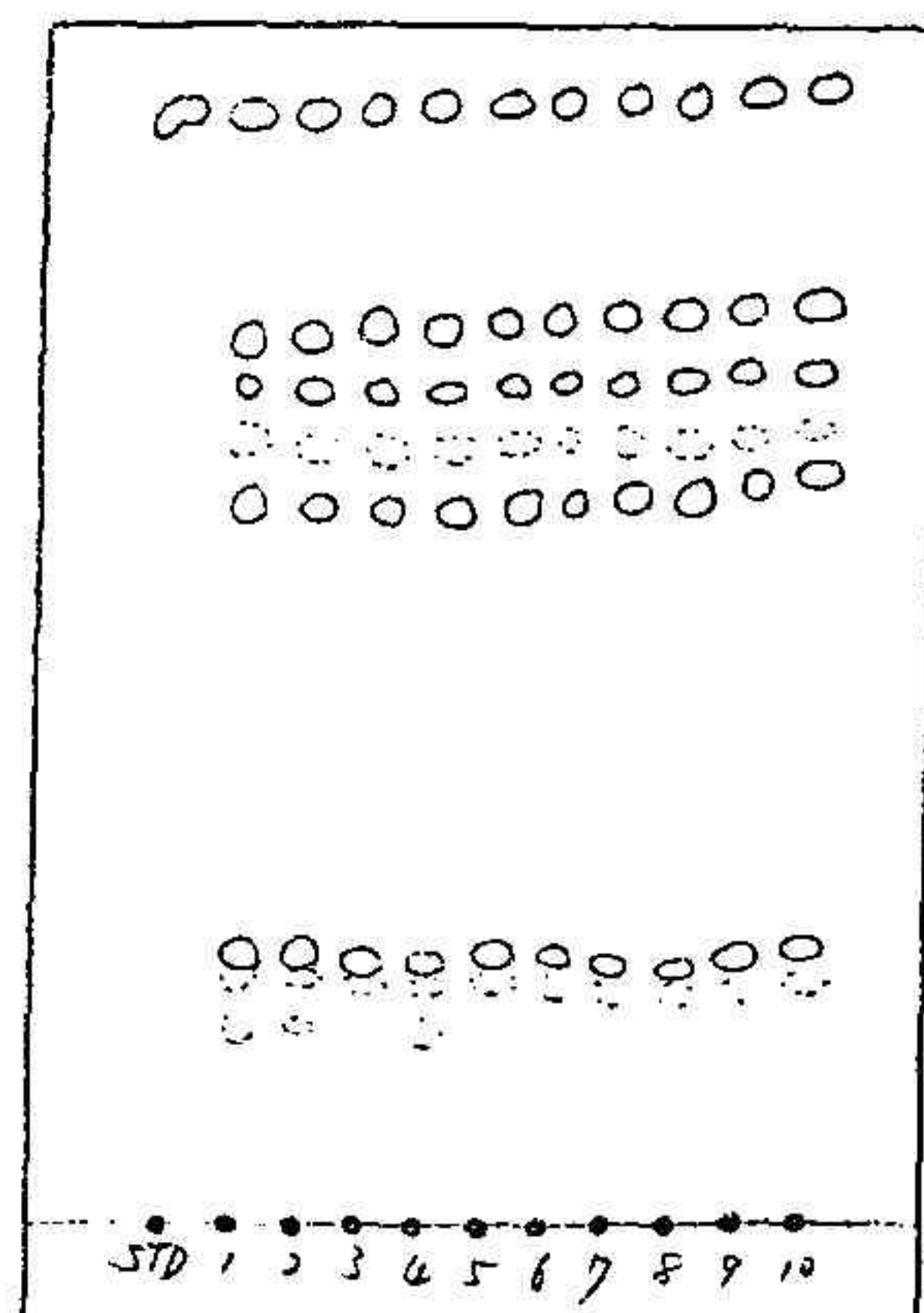
STD₂: Saikosaponin-a

STD₃: Saikosaponin-c

Solvent system :EA:MeOH :H₂O=10:2:1

Sprayer : 10% H₂SO₄

大黃 *Rhei Rhizoma*

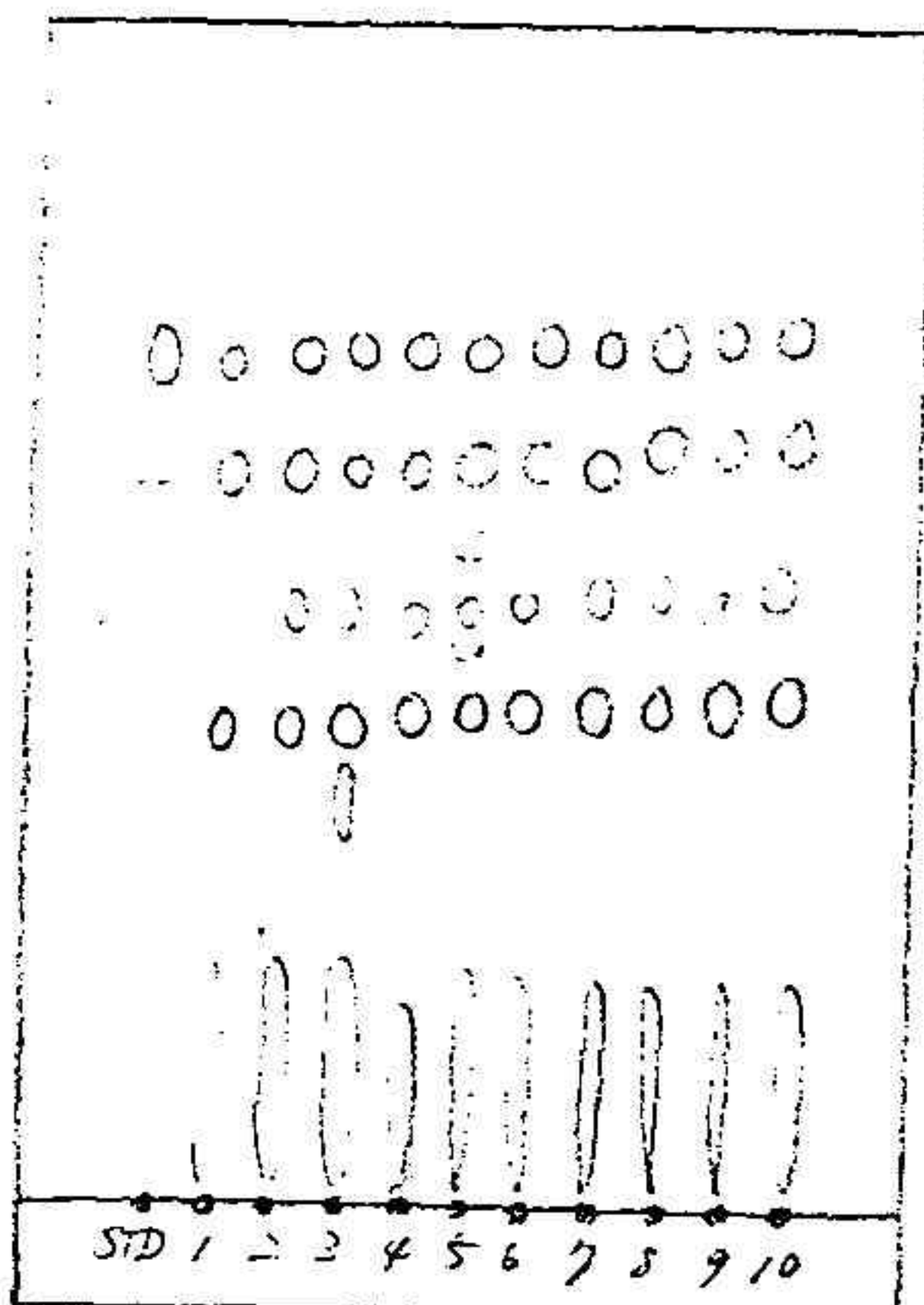


STD: emodin

Solvent system: EtOAc:MeOH:H₂O= 10: 2: 1

Sprayer: 1% FeCl₃/MeOH

玄参 Scrophulariae Radix

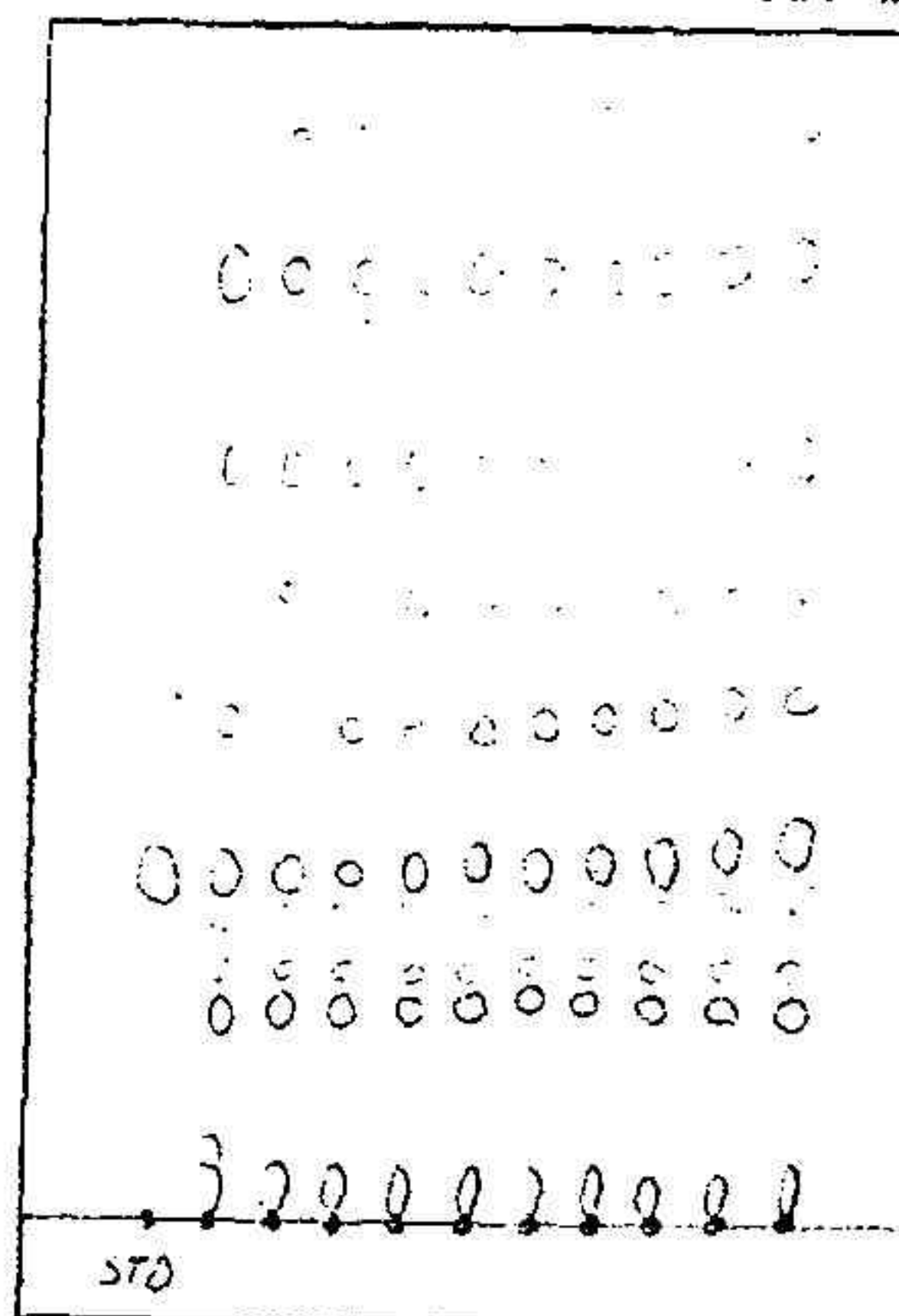


STD: harpagoside

Solvent system: n-butanol:acetic acid:H₂O
=4: 1: 5

Sprayer: p-anisaldehyde/H₂SO₄

苍朮 Atractylodis Lanceae Rhizome

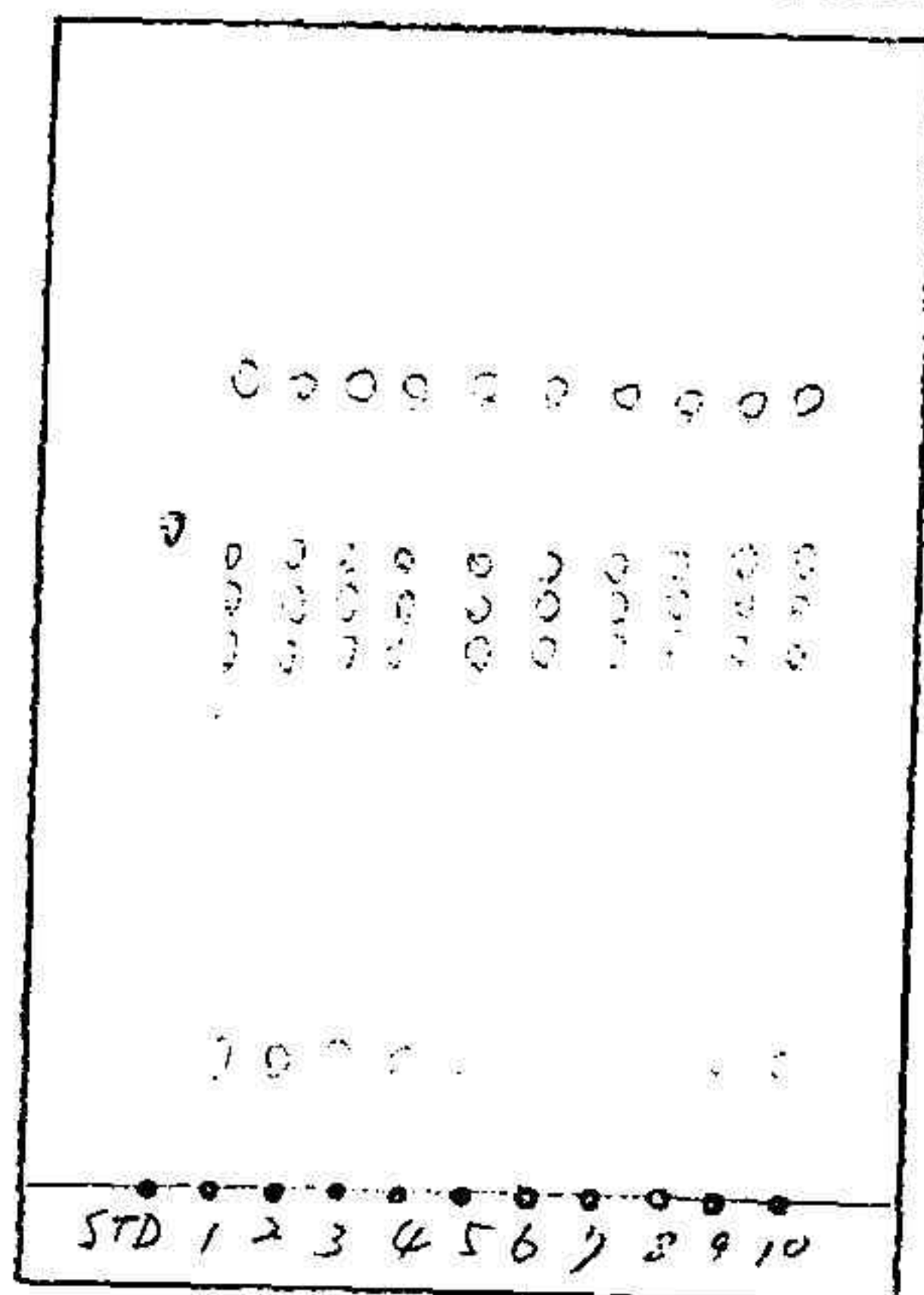


STD: b-eudesmol

Solvent system: n-hexane: EtOAc= 9: 1

Sprayer: p-anisaldehyde/H₂SO₄

北茵陈 Origanum Vulgare Herba

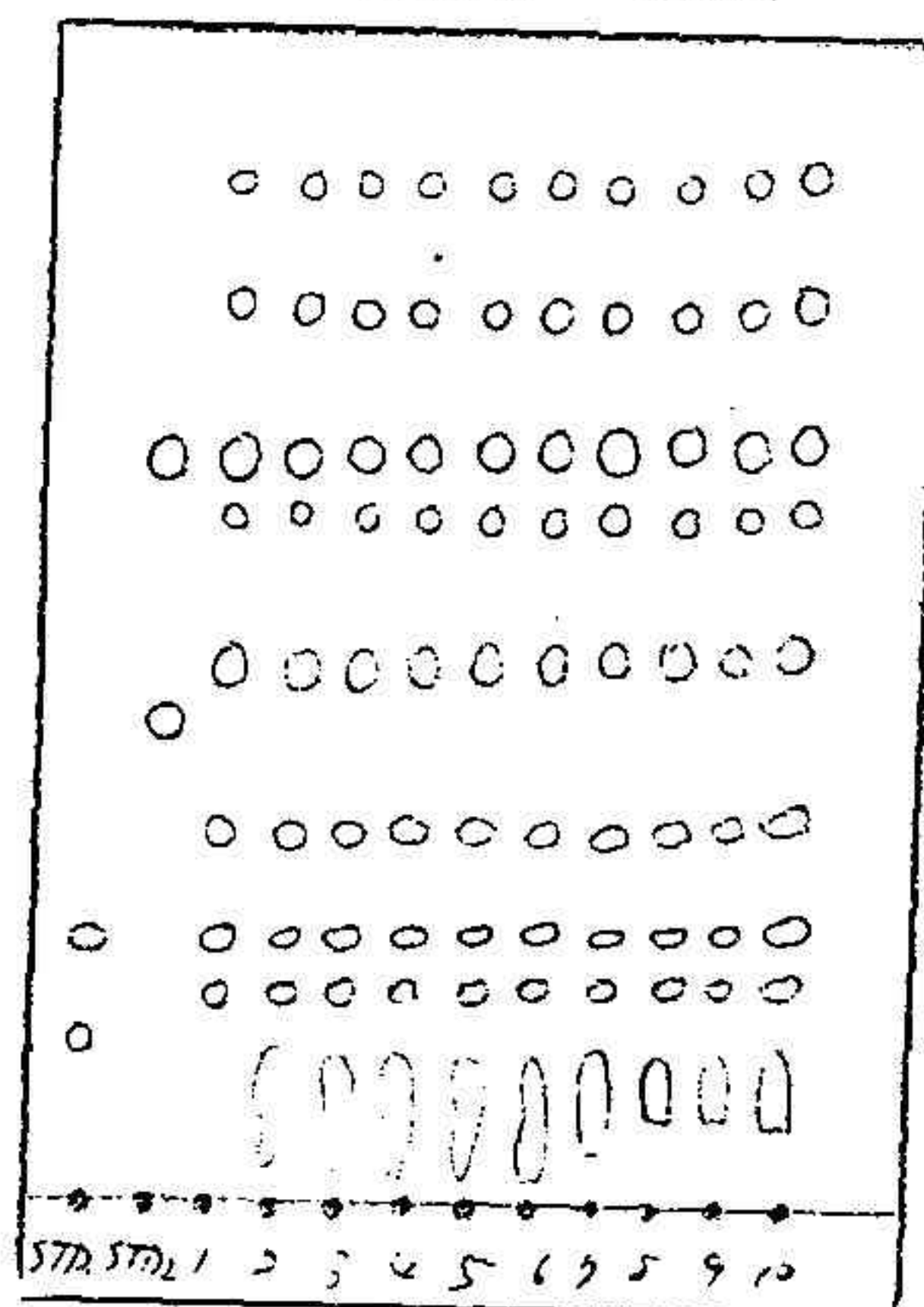


STD: capillarisin

Solvent system: n-hexane: CHCl₃:EtOAc
=4: 1 :1

Sprayer: 10% H₂SO₄

人参 Ginseng Radix

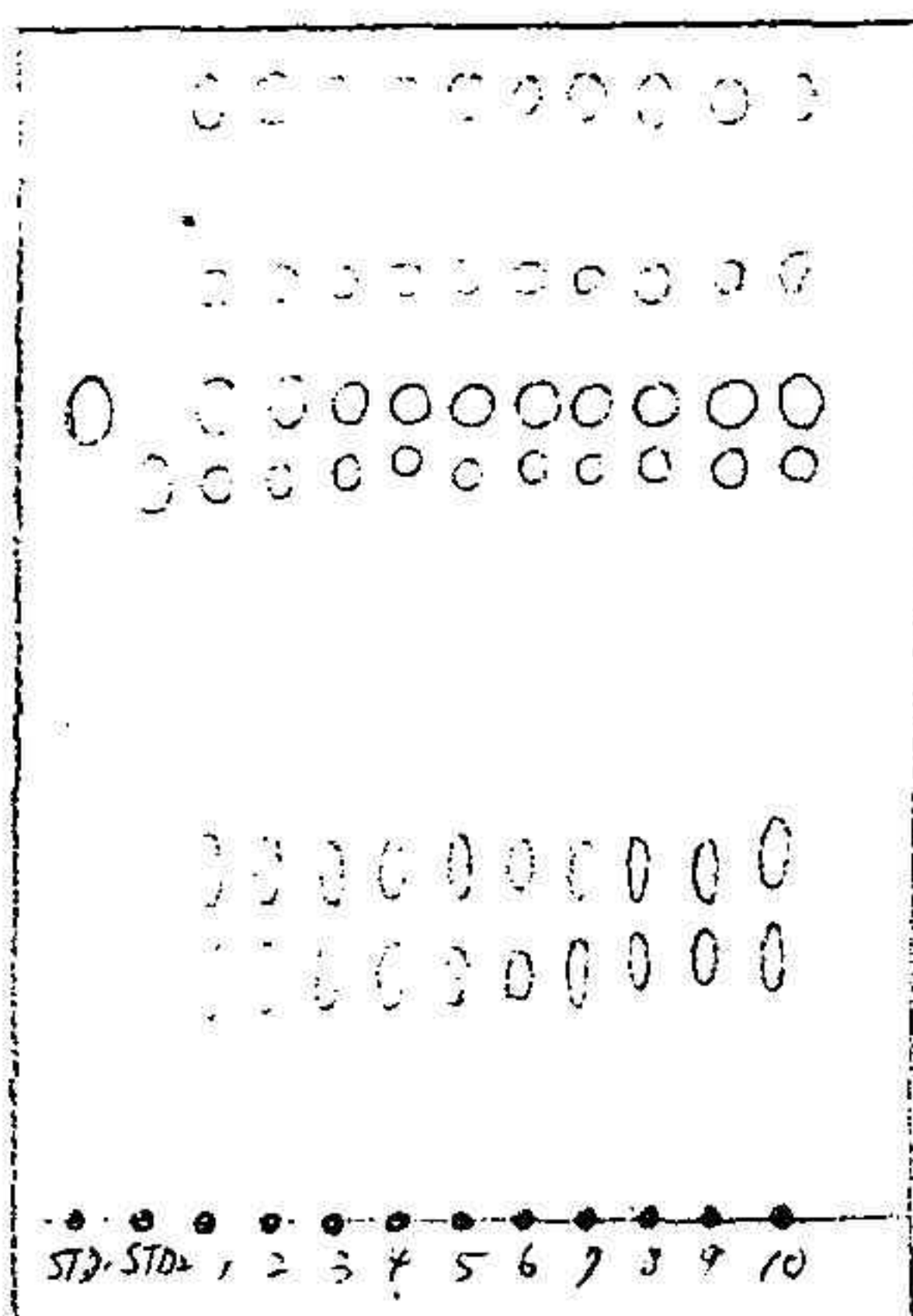


STD₁: ginsenoside R_{g1} STD₂: ginsenoside R_{g2}

Solvent system: n-butanol:acetic acid:H₂O=7:1:2

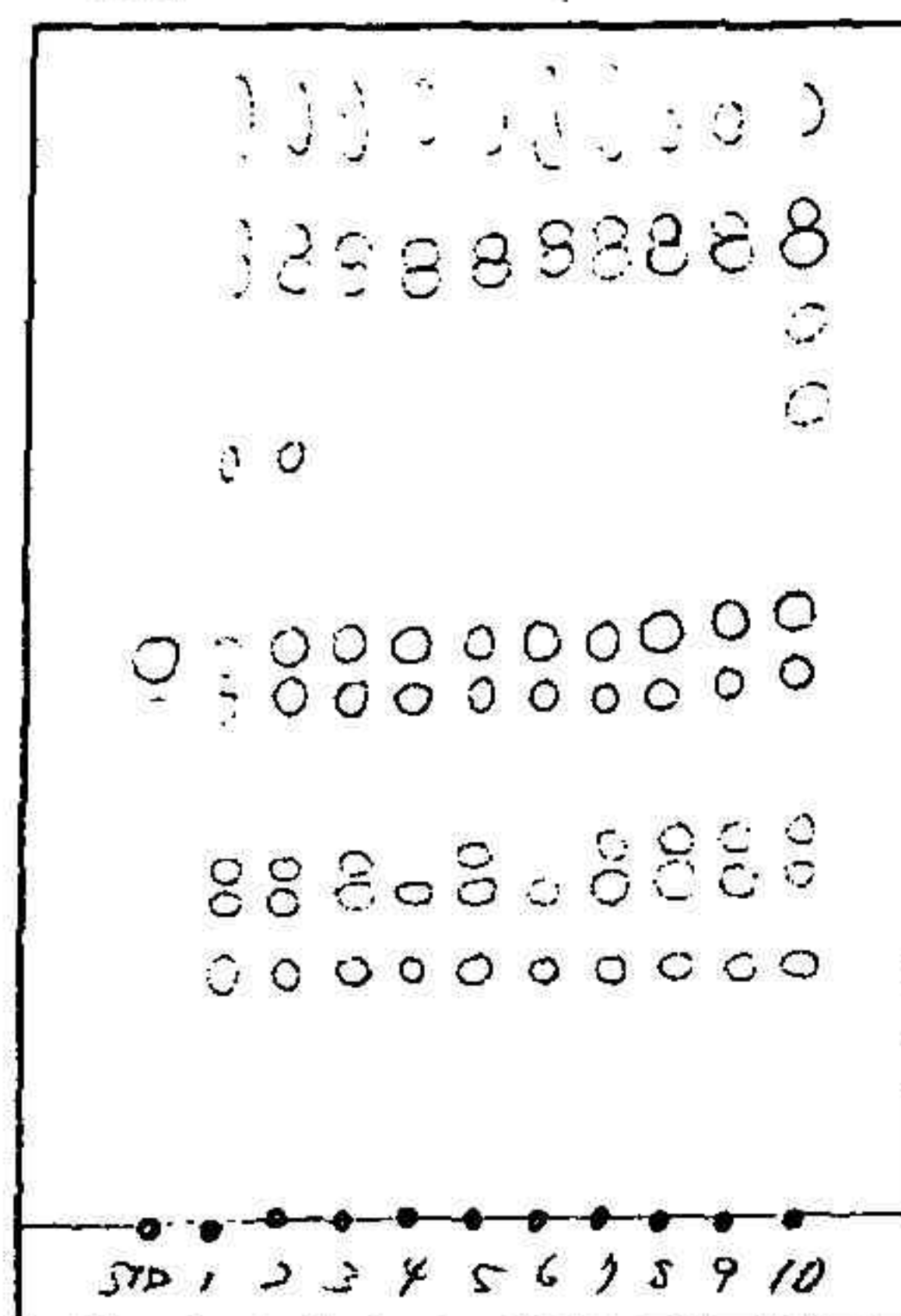
Sprayer: 10% H₂SO₄

厚朴 Magnoliae Cortex



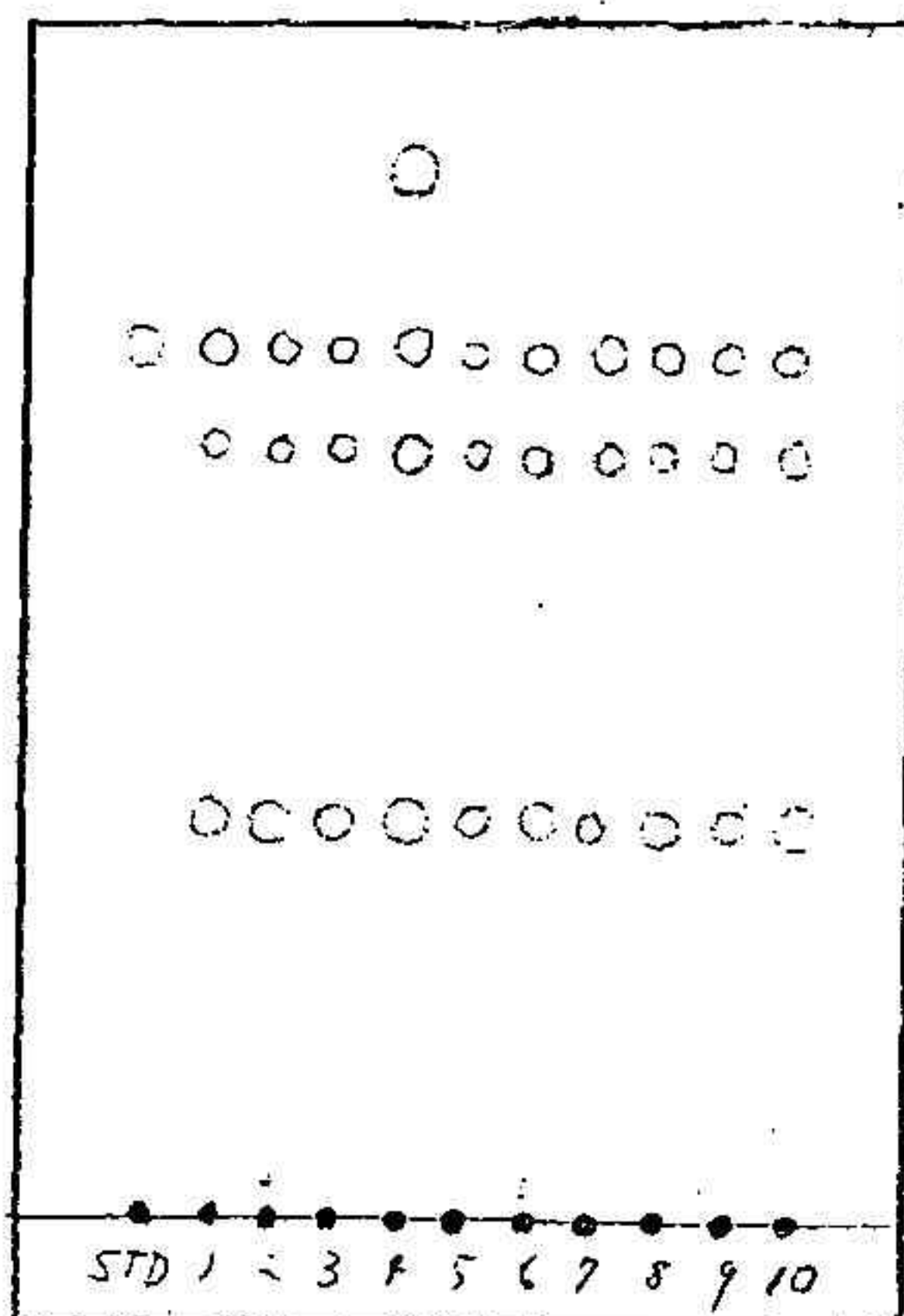
STD₁: honokiol STD₂: magnolol
Solvent system: CHCl₃:EA=6:1
Detection: UV

黄柏 Phellodendri Cortex



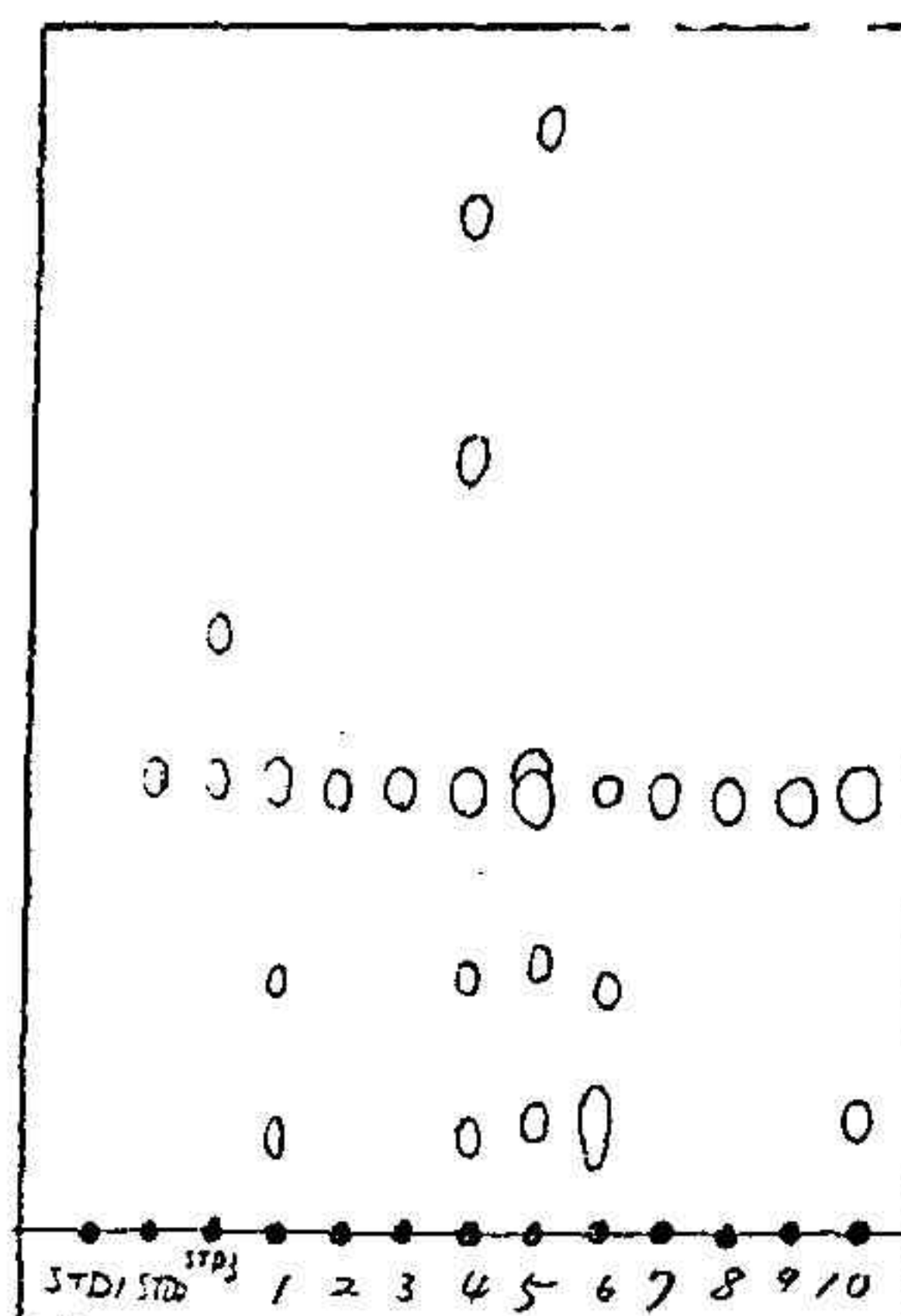
STD: berberine
Solvent system: n-butanol: acetic acid:H₂O
=7:1:2
Detection: UV, Dragendorff

芍药 Paeoniae Albiflorae Radix



STD: paeoniflorin
Solvent system: n-butanol:acetic acid:H₂O
=7:1:2
Sprayer: p-anisaldehyde/H₂SO₄

葛根 Puerariae Radix



STD₁: diazein STD₂: daizein
STD₃: puerarin
Solvent system: toluene: CHCl₃:acetone
=40:25:35
Detection: UV, 1% FeCl₃-MeOH

四、討論：

- 1.本計畫針對市售 20 種中藥，每種取 10 個樣品進行 TLC 及 HPLC 分析，由 HPLC 分析結果，顯示大部份藥材中指標成分的含量有很大的差異，此可能由於進口藥材生長，採收時間不同，以及品種或代用品不同所致。
- 2.茵陳由於市售藥材有兩種，北茵陳及綿茵陳，前者屬唇形科，後者屬菊科，經 HPLC 分析兩種所含成分也不同，而無論就 TLC 或 HPLC 分析顯示兩者均不含目前一般所使用之指標成分 capillarisin.
- 3.部份藥材如蒼朮及牡丹皮所含成分具揮發性，本計畫中所使用之萃提方式是熱萃取，其含量可能會有漏失，此外或許可考慮以 GC 取代 HPLC 進行定量。
- 4.部份指標成分純度不佳，雜質含量多，造成 HPLC 分析上指標成分峰之確認困難，同時發生定量誤差及難以定量等問題，值得當局注意。