QUALITY ASSESSMENT OF *ARDISIA ELLIPTICA* FRUIT AND ANALYSIS OF EMBELIN CONTENT BY THIN LAYER CHROMATOGRAPHIC TECHNIQUES

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การประเมินมาตรฐานของผลพิลังกาสาและวิเคราะห์ปริมาณเอ็มบิลินโดยเทคนิคทางทินเลเยอร์โครมาโทกราฟี

นายพงศธร อยู่คงพัน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	QUALITY ASSESSMENT OF *ARDISIA ELLIPTICA* FRUIT AND ANALYSIS OF EMBELIN CONTENT BY THIN LAYER CHROMATOGRAPHIC TECHNIQUES
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พงศธร อยู่คงพัน : การประเมินมาตรฐานของผลพิลังกาสาและวิเคราะห์ปริมาณเอ็มบิลิน โดยเทคนิคทางทินเลเยอร์โครมาโทกราฟี (QUALITY ASSESSMENT OF *ARDISIA ELLIPTICA* FRUIT AND ANALYSIS OF EMBELIN CONTENT BY THIN LAYER CHROMATOGRAPHIC TECHNIQUES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร. ชนิดา พลานุเวช อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร. นิจศิริ เรืองรังษี, 116 หน้า.

พิลังกาสา มีชื่อทางวิทยาศาสตร์ว่า *Ardisia elliptica* Thunb. พิลังกาสาเป็นเครื่องยาสมุนไพรที่ใช้ในตำรับยาไทย เช่น อภัยสาลี ปลูกไฟธาตุ และปราบชมพูทวีป การศึกษานี้มีจุดประสงค์เพื่อจัดทำข้อกำหนดทางเภสัชเวทของผลพิลังกาสาในประเทศไทย รวมทั้งวิเคราะห์หาปริมาณสารสำคัญเอ็มบิลินในผลพิลังกาสาโดยวิธีทินเลเยอร์โครมาโทกราฟี-เด็นซิโทเมทรี และ การวิเคราะห์รูปภาพทางทินเลเยอร์โครมาโทกราฟี โดยศึกษาผลพิลังกาสาจาก 15 แหล่งทั่วประเทศไทย วาดภาพลายเส้นแสดงลักษณะทั้งต้นของพิลังกาสา เตรียมเครื่องยาให้สะอาดและอบแห้ง ลักษณะทางมหภาคของเครื่องยามีรูปร่างเป็นผลแห้ง สีน้ำตาลเข้ม ภายในมีเมล็ดสีน้ำตาล มีกลิ่นหอม ลักษณะเด่นทางจุลภาคของผลพิลังกาสาคือ เซลล์หิน ท่อลำเลียงอาหาร เนื้อเยื่อต้นอ่อน เซลล์ผิวของเมล็ด การศึกษาเอกลักษณ์ทางเคมี-ฟิสิกส์พบว่า มีปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ความชื้น ปริมาณสารสกัดด้วยเอทานอล ปริมาณสารสกัดด้วยน้ำ และปริมาณน้ำ เท่ากับ 5.57 ± 0.15, 0.34 ± 0.06, 10.54 ± 0.08, 4.92 ± 0.45, 9.42 ± 0.91, 10.61 ± 0.69 โดยน้ำหนัก ตามลำดับ การศึกษาด้วยเทคนิคทางทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลายคลอโรฟอร์ม เอทิลอะซิเทต และ กรดฟอร์มิค (5:4:1) เป็นเฟสเคลื่อนที่ ตรวจจับภายใต้แสงอุลตราไวโอเล็ต (254 นาโนเมตรและ365 นาโนเมตร) เช่นเดียวกับฉีดพ่นด้วยน้ำยากรดฟอสโฟโมลิบดิก พบแถบที่โดดเด่นที่ค่า Rf เท่ากับ 0.35 การวิเคราะห์เชิงปริมาณด้วยเทคนิคทางทินเลเยอร์โครมาโทกราฟี โดยใช้ตัวทำละลาย เอ็น-บิวทานอล เอ็น-โพรพานอล 4นอร์มอลแอมโมเนีย (1:7:2) เป็นเฟสเคลื่อนที่ วิเคราะห์สารเอ็มบิลินโดยวิธีทินเลเยอร์โครมาโทกราฟี-เด็นซิโทเมทรี มีช่วงความเป็นเส้นตรงระหว่าง 0.60 – 3.00 ไมโครกรัม และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.992 ขีดจำกัดของการตรวจพบและขีดจำกัดของการหาปริมาณ มีค่า 21.04 และ 63.76 นาโนกรัม ระดับความเที่ยงของวิธีวิเคราะห์ ประเมินจากค่าสัมประสิทธิ์ของการกระจาย มีค่าระหว่างร้อยละ 8.55 – 18.74 ค่าเฉลี่ยการคืนกลับระหว่างร้อยละ 82.88 – 103.00 วิเคราะห์สารเอ็มบิลินโดยวิธีการวิเคราะห์รูปภาพทางทินเลเยอร์โครมาโทกราฟี มีช่วงความเป็นเส้นตรงระหว่าง 0.60 – 3.00 ไมโครกรัม และมีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ 0.994 ขีดจำกัดของการตรวจพบและขีดจำกัดของการหาปริมาณ มีค่า 8.05 และ 24.40 นาโนกรัม ระดับความเที่ยงของวิธีวิเคราะห์ ประเมินจากค่าสัมประสิทธิ์ของการกระจาย มีค่าระหว่างร้อยละ 1.92 – 9.29 ค่าเฉลี่ยการคืนกลับระหว่างร้อยละ 103.83 – 123.50 ปริมาณสารเอ็มบิลินในผลพิลังกาสาโดยวิธีทินเลเยอร์โครมาโทกราฟี-เด็นซิเด็นซิโทเมทรี และ การวิเคราะห์รูปภาพทางทินเลเยอร์โครมาโทกราฟีมีค่าระหว่าง 0.20 - 4.53 และ 0.23 - 4.43 กรัม/ 100 กรัมของผลแห้ง ตามลำดับ การเปรียบเทียบปริมาณเอ็มบิลินระหว่าง 2 วิธี ถูกทดสอบโดยใช้สถิติ Wilcoxon Signed Ranks Test พบว่าปริมาณเอ็มบิลินโดยสองวิธีไม่แตกต่างกัน (Z =-1.761, P = 0.078)ผลการศึกษาครั้งนี้สามารถจัดทำเป็นข้อกำหนดมาตรฐานของสมุนไพรพิลังกาสาในประเทศไทย ซึ่งจะเป็นประโยชน์ต่อการควบคุมคุณภาพและความปลอดภัยในการใช้เครื่องยานี้

ภาควิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : PHARMACOGNOSTIC SPECIFICATION / *ARDISIA ELLIPTICA* / EMBELIN / THIN LAYER CHROMATOGRAPHY-DENSITOMETRY/ THINLAYER CHROMATOGRAPHY IMAGE ANALYSIS

PONGSATHORN YUKONGPHAN : QUALITY ASSESSMENT OF *ARDISIA ELLIPTICA* FRUIT AND ANALYSIS OF EMBELIN CONTENT BY THIN LAYER CHROMATOGRAPHIC TECHNIQUES. ADVISOR : CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 116 pp.

Ardisia elliptica Thunb. dried fruits has been traditionally used as crude drug in Thai remedies such as Apaisali, Plookfietad and Prabchomphuthaveeb. This study aimed to report the current information on the pharmacognostic specification of *A. elliptica* dried fruits and analysed the chemical constituent, embelin by thin layer chromatography-densitometry and thin layer chromatography image analysis. The fruits were collected from 15 habitats located at various regions throughout Thailand. The whole plant of *A. elliptica* was illustrated in detail. The crude drug was prepared by cleaning, and drying in a hot air oven. The macroscopic characters were dried fruits with calyx, variable in sizes, dark brown and brown seeds. The anatomical and histological characteristics were sclereids or stone cell, recticulated vessels, endosperm, epidermis of seed. The total ash, acid insoluble ash, loss on drying, ethanol–soluble extractive, water–soluble extractive and water content of 5.57 ± 0.15, 0.34 ± 0.06, 10.54 ± 0.08, 4.92 ± 0.45, 9.42 ± 0.91 and 10.61 ± 0.69 % dry weight respectively. Thin-layer chromatographic fingerprints of ethanolic extracts of *A. elliptica* dried fruits were studied using chloroform, ethyl acetate and formic acid (5:4:1) as mobile phase. Detection under ultraviolet light (254 nm and 365 nm) as well as spraying with phosphomolypdic acid reagent showed the dominant band at R*f* = 0.35. Thin layer chromatography for quantitative analysis was studied using n-butabol, n-propanol, 4N ammonia (1:7:2) as mobile phase. Linearity range of embelin by TLC-densitometry was 0.60 - 3.00 µg with correlation coefficient (r^2) of 0.992, LOD and LOQ were 21.04 and 63.76 ng respectively. The precision was evaluated by the % RSD of repeatability and intermediate precision, was between 8.83 – 12.45 % RSD and 8.55 – 18.74 %RSD respectively. The average recoveries were 82.88 – 103.00 % recoveries. Linearity range of embelin by TLC-image analysis was 0.60 - 3.00 µg with correlation coefficient (r^2) of 0.994, LOD and LOQ were 8.05 and 24.40 ng respectively. The precision was evaluated by the % RSD of repeatability and intermediate precision, was between 5.27 – 8.12 % RSD and 1.92 – 9.29 %RSD respectively. The average recoveries were between 103.83 – 123.50 % recoveries. The quantitative results showed that embelin content in *A. elliptica* fruits by TLC-densitometry were between 0.20 – 4.53 g/100 g of dried fruit whereas by TLC image analysis was found at 0.23 – 4.43 g/100 g of dried fruit. The comparison of embelin content between TLC densitometry and TLC image analysis were statistically tested using Wilcoxon Signed Ranks Test. It was found that the embelin contents by two methods were not significantly different ($Z = -1.761$, $P = 0.078$). This study provides scientific information for the quality control of *A. elliptica* dried fruits including embelin content in Thailand that leads to safe use of this crude drug.

Field of Study : Public Health Sciences ..... Student's Signature .....

Academic Year : 2012 ..... Advisor's Signature .....

Co-advisor's Signature .....

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G	=	Gram
GC	=	Gas chromatography
HPLC	=	High performance liquid chromatography
HPTLC	=	High performance thin layer chromatography
ICH	=	The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
l	=	liter
mg	=	milligram
ml	=	milliliter
ng	=	nanogram
nm	=	nanometer
r^2	=	Correlation coefficients
Rf	=	Retention factors
RSD	=	Relative standard deviation
SD	=	Standard deviation
TLC	=	Thin layer chromatography
WHO	=	World Health Organizations
µg	=	microgram
µl	=	microliter
α	=	Alpha
β	=	Beta

CHAPTER I

INTRODUCTION

Background and significance of the study

In the last decades herbal medicines have been popular and developmental in many countries around the world since the World Health Organization urged its member countries to use folk healing practices and herbal medicines as part of the basic public health projects. The Herbal medicines are u sed as remedies, over-the-counter drug products and raw materials for the pharmaceutical industry. However the quality of herbal medicine has not been shown enough to confirm the confidence of consumer. Therefore there are needs of the procedures to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards by following the World Health Organization guidelines [1].

In Thailand, herbal medicines have been used for a long time and the popularity of herbal medicines has been greatly increased in the last few years. According to the National List of Essential Medicines 2011 (the current version), there are a lot of medicinal plants and herbal drugs published. All of these drugs and medicinal plants are greatly used to treat and relieve the diseases. Nevertheless they are not only used in commercial market but also used widely in the hospital. In 2010, one study showed that there were a lot of factors affected to determine the usage of herbal medicines such as inadequate scientific researches, low confidence of physicians to use herbal medicine and the appropriate drug use [2].

Ardisia elliptica Thunb. (Myrsinaceae), an evergreen shrub with smooth stem and new foliage often reddish, also known as Pilangkasa is one of the herbs in Thai traditional medicine. It is mainly used for treatment of the diseases such as diarrhea, fever, liver disease, and leprosy disease [3].

Ardisia elliptica fruits contain a quinone derivative, embelin as a major constituent. Myricetin, quercetin, berginin, norbergenin, kaempferol, quercetin 3-*O*-*β* - d -glucopyranoside and gallic acid were reported as well [4].

Even though *Ardisia elliptica* fruit is widely used in tradition medicine, and it is safe and efficacious to use but there are no standardization parameters to justify the quality. Thin layer chromatography is a method for screening plant extracts. This method is very easy, rapid and cheap methods for screening the active compound in plant extract. This study aims to report the current information on the phamacognostic specifications of *Ardisia elliptica* fruits and the embelin contents in each sample.

Objectives

1. To develop the standardization parameters of *Ardisia elliptica* fruits.
2. To investigate the contents of embelin in *Ardisia elliptica* fruits.
3. To compare the method validation between TLC image analysis by ImageJ free software and TLC densitometry.

Expected Benefit & Application

1. This research will provide the standardization parameters of *Ardisia elliptica* fruits.
2. This research will provide the contents of embelin in *Ardisia elliptica* fruits.
3. This research will provide the simple, less expensive and valid method of TLC image analysis for embelin quantitation in *Ardisia elliptica* fruits.

Conceptual framework

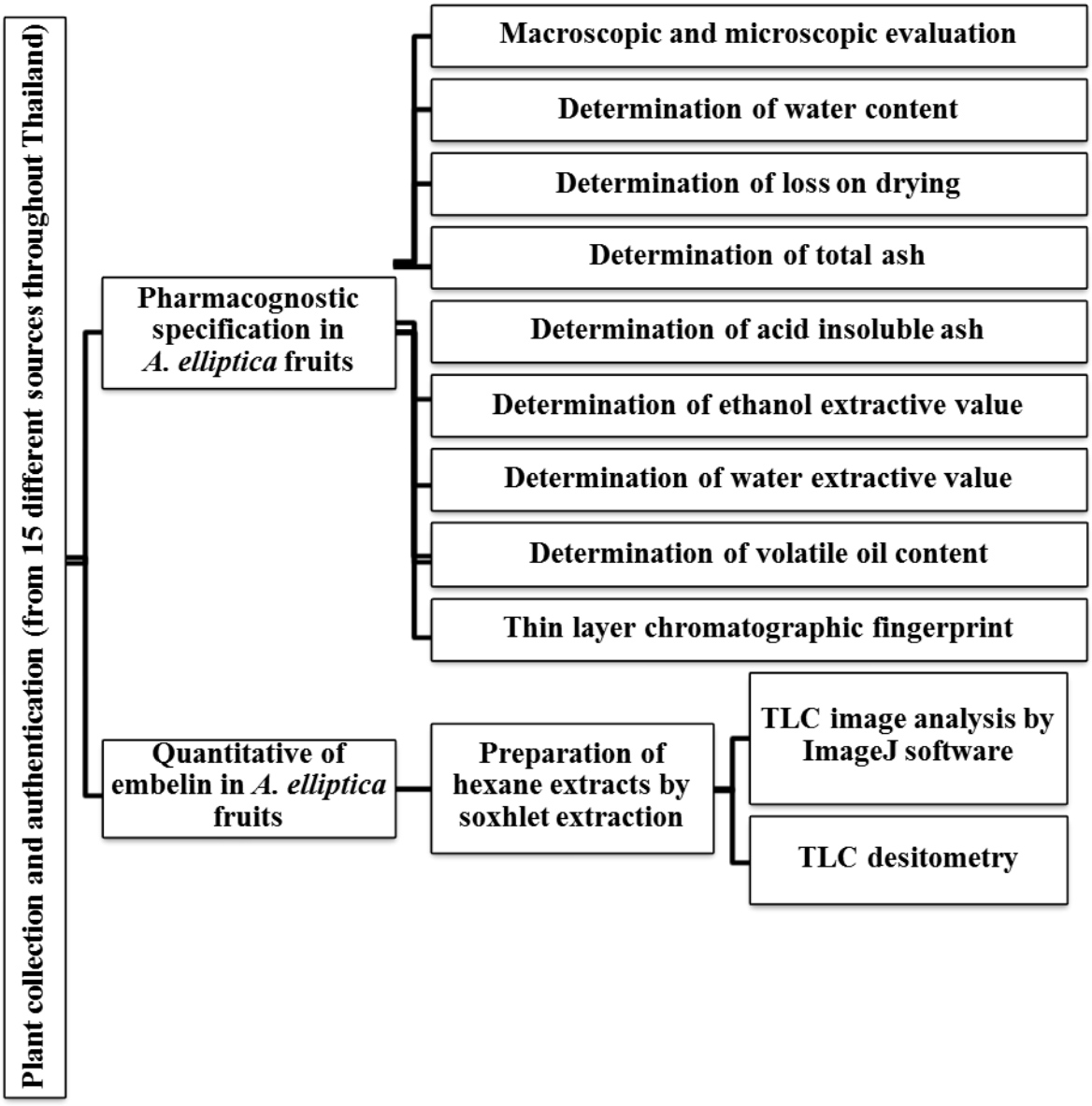


Figure 1. The conceptual framework

CHAPTER II

REVIEW LITERATURE

Genus *Ardisia*

The plants of the genus *Ardisia* are commonly used in folk medicine around the world because of their phytotherapeutic secondary metabolites. The genus *Ardisia* belongs to the family Myrsinaceae. There are 6 genus of Myrsinaceae reported in Thailand (Table 2) namely *Aegiceras*, *Ardisia*, *Embelia*, *Labisia*, *Maesa* and *Rapanea* [Thai Plant Name]. Genus *Ardisia* is the largest which contains 48 species [5,6].

Previous researches revealed the medicinal properties of *Ardisia* (Table 3). Several groups of biologically active phytochemicals were reported including saponins, coumarins, quinones and alkylphenols. The phytochemical constituents of *Ardisia* species and plant parts used for isolation of compounds are shown in table 4 [3]. According to this unique phytochemical composition in *Ardisia* species, it has a potential for phytotherapeutic as a source of novel agents, especially embelin, a benzoquinone found in Myrsinaceae [3].

Table 1. Description of family Myrsinaceae [7]

Plant	Herbs, trees or shrubs, occasionally climbers
Leaf	Opposite, alternate or whorled, sometimes 2-ranked, simple, mostly entire, often leathery, usually glandular-punctate, dots coloured or ± translucent; stipules absent.
Inflorescence	Terminal, axillary or ramiflorous, raceme-like, paniculate or in ± sessile umbellate clusters or flowers solitary and axillary. Flowers actinomorphic, 4- or 5-merous, mostly bisexual, sometimes bracteate, rarely bracteolate. Calyx usually deeply lobed, persistent, often glandular. Corolla often deeply lobed, sometimes free, not persistent. Stamens opposite to and as many as the corolla lobes or petals, dehiscence introrse by longitudinal slits or by apical pores. Gynoecium of 3–5 carpels united to form a 1-locular, superior ovary or rarely half-inferior (Samolus); style simple; ovules 1-numerous.
Fruit	A drupe, capsule or berry; seeds 1-many.
Occurrence:	World: c. 50 genera, c. 1500 species

Table 2. Family Myrsinaceae in Thailand [6]

Aegiceras **(1 Species)**	***Embelia*** **(14 Species)**	***Labisia*** **(1 Species)**	***Maesa*** **(10 Species)**	***Rapanea*** **(3 Species)**
Aegiceras corniculatum (L.) Blanco	*Embelia coriacea* Wall. ex A.DC. *Embelia grandifolia* Fletcher *Embelia impressa* Fletcher *Embelia kerrii* Fletcher *Embelia macrocarpa* King & Gamble *Embelia oblongifolia* Hemsl. *Embelia pulchella* Mez *Embelia ribes* Burm.f. *Embelia scandens* (Lour.) Mez *Embelia sessiliflora* Kurz *Embelia sootepensis* Craib *Embelia subcoriacea* (C.B.Clarke) Mez *Embelia tsjeriamcottam* (Roem. & Schult.) A.DC. var. *ferruginea* (C.B.Clarke) K.Larsen & C.M.Hu *Embelia tsjeriamcottam* (Roem. & Schult.) A.DC. var. *tsjeriamcottam*	*Labisia pumila* (Blume) Fern.-Vill. & Naves	*Maesa argyrophylla* K.Larsen & C.M.Hu *Maesa glomerata* K.Larsen & C.M.Hu *Maesa indica* (Roxb.) A.DC. *Maesa integrifolia* Ridl. *Maesa lineolata* H.R.Fletcher *Maesa montana* A.DC. *Maesa paniculata* A.DC. *Maesa perlarius* (Lour.) Merr. *Maesa permollis* Kurz *Maesa ramentacea* (Roxb.) A.DC.	*Rapanea porteriana* (A.DC.) Mez *Rapanea umbellulata* (A.DC.) Mez *Rapanea yunnanensis* Mez

Table 2 (Cont.). Family Myrsinaceae in Thailand [6]

***Ardisia* (48 Species)**		
Ardisia alata Fletcher	*Ardisia elliptica* Thunb.	*Ardisia porosa* C.B.Clarke
Ardisia amherstiana A.DC. var. *amherstiana*	*Ardisia fulva* King & Gamble var. ciliata Fletcher	*Ardisia puberula* Fletcher
Ardisia amherstiana A.DC. var. *pubescens* (Fletcher) K.Larsen & C.M.Hu	*Ardisia fulva* King & Gamble var. fulva	*Ardisia quinquegona* Blume
Ardisia aprica Fletcher	*Ardisia furva* Ridl.	*Ardisia ridleyi* King & Gamble
Ardisia atrovirens K.Larsen & C.M.Hu	*Ardisia helferiana* Kurz	*Ardisia rigida* Kurz var. laevis K.Larsen & C.M.Hu
Ardisia attenuata Wall. ex A.DC.	*Ardisia impressa* Fletcher var. grandidens K.Larsen & C.M.Hu	*Ardisia rigida* Kurz var. rigida
Ardisia betongensis Fletcher	*Ardisia impressa* Fletcher var. impressa	*Ardisia rosea* King & Gamble
Ardisia bractescens Ridl.	*Ardisia ionantha* K.Larsen & C.M.Hu	*Ardisia sumatrana* Miq.
Ardisia collinsae Fletcher	*Ardisia lanceolata* Roxb.	*Ardisia symplocifolia* (C.Chen) K.Larsen & C.M.Hu
Ardisia colorata Roxb.	*Ardisia longipedicellata* Fletcher	*Ardisia tetramera* K.Larsen & C.M.Hu
Ardisia confusa K.Larsen & C.M.Hu	*Ardisia maculosa* Mez	*Ardisia tuberculata* Wall. ex A.DC.
Ardisia congesta Ridl.	*Ardisia murtonii* Fletcher	*Ardisia undulatodentata* Fletcher
Ardisia corymbifera Mez var. *corymbifera*	*Ardisia oxyphylla* Wall. ex A.DC	*Ardisia uniflora* K.Larsen & C.M.Hu
Ardisia corymbifera Mez var. *euryoides* K.Larsen & C.M.Hu	*Ardisia palustris* K.Larsen & C.M.Hu	*Ardisia villosa* Roxb.
Ardisia crenata Sims var. *angusta* C.B.Clarke	*Ardisia pilosa* Fletcher	*Ardisia virens* Kurz
Ardisia crenata Sims var. *crenata*	*Ardisia polycephala* Wall. ex A.DC.	*Ardisia wallichii* A.DC.

Table 3. Medicinal properties of *Ardisia* species [5]

***Ardisia* species**	**Medicinal properties**	**Plant parts used**
Ardisia arborescens Wall. ex A. DC	Febrifuge	NS
Ardisia colorata Roxb.	Liver disease, cough, and diarrhea	NS
	Antioxidant	Fruits
Ardisia compressa Kunth.	Various liver aliments	Leaves
	Antioxidants and antitumor activities	NS
Ardisia cornudentata Mez.	Anti-inflammatory and analgesic	Whole plant
Ardisia crenata Sims.	Utero-contraction	Rhizomes
	Platelet aggregation and blood pressure lowering	Whole plant
	cAMP inhibition	Roots
	Antithrombin activity	NS
Ardisia crispa (Thunb.) A.DC.	Antimetastatic and antitumor	NS
Ardisia elliptica Thunb.	Alleviating chest pain	NS
	Antibiotic (as *Ardisia solanacea*)	Leaves
	Antiviral (as *Ardisia squamulosa*)	Stems and leaves
	Birth complications, fever, diarrhea, liver poisoning	NS
	Gonorrhea and other venereal diseases	NS
	Antiproliferative activity against human breast adenocarcinoma (SKBR3) cells	Fruits
Ardisia iwahigensis Elmer	Cytotoxic	Twigs and leaves
Ardisia japonica (Thunb.) Blume	Anticancer (pancreatic and other types)	Whole plant
	Anti-HIV	Aerial parts
	Antioxidant	Whole plant
	Inhibitors of the human protein tyrosine phosphatase 1B	NS
	5-Lipoxygenase inhibition and antiallergenic	Rhizomes
	Type 2 diabetes mellitus	Whole plant

NS = Not specified

Table 3 (Cont.). Medicinal properties of *Ardisia* species [5]

***Ardisia* species**	**Medicinal properties**	**Plant parts used**
Ardisia maculosa Mez.	Antibiotic	Whole plant
	Heptoprotector	NS
Ardisia mamillata Hance	Respiratory tract infection and menstrual disorder	Roots
Ardisia pusilla A.DC.	Immunological function and anti-tumor activity	NS
Ardisia sieboldii Miq.	Hepatoprotector	NS
	5-Lipoxygenase inhibition	Bark
Ardisia silvestris Pit	Colic and stomachache	Branches and leaves
Ardisia solanacea Roxb.	To treat asthma	Leaves and flowers
	Febrifuge	Root bark
Ardisia squamulosa Presl	Antiviral against herpes simplex and adenoviruses	Stem and leaves
Ardisia teysmanniana Scheff.	Antimicrobial	Leaves
Ardisia villosa Roxb.	Analgesic	Whole plant
	Febrifuge and antitussive	Roots

NS = Not specified

Table 3 (Cont.). Medicinal properties of *Ardisia* species [5]

***Ardisia* species**	**Medicinal properties**	**Plant parts used**
Ardisia arborescens Wall. ex A. DC	Febrifuge	NS
Ardisia colorata Roxb.	Liver disease, cough, and diarrhea	NS
	Antioxidant	Fruits
Ardisia compressa Kunth.	Various liver aliments	Leaves
	Antioxidants and antitumor activities	NS
Ardisia cornudentata Mez.	Anti-inflammatory and analgesic	Whole plant
Ardisia crenata Sims.	Utero-contraction	Rhizomes
	Platelet aggregation and blood pressure lowering	Whole plant
	cAMP inhibition	Roots
	Antithrombin activity	NS
Ardisia crispa (Thunb.) A.DC.	Antimetastatic and antitumor	NS
Ardisia elliptica Thunb.	Alleviating chest pain	NS
	Antibiotic (as *Ardisia solanacea*)	Leaves
	Antiviral (as *Ardisia squamulosa*)	Stems and leaves
	Birth complications, fever, diarrhea, liver poisoning	NS
	Gonorrhea and other venereal diseases	NS
	Antiproliferative activity against human breast adenocarcinoma (SKBR3) cells	Fruits
Ardisia iwahigensis Elmer	Cytotoxic	Twigs and leaves
Ardisia japonica (Thunb.) Blume	Anticancer (pancreatic and other types)	Whole plant
	Anti-HIV	Aerial parts
	Antioxidant, Type 2 diabetes mellitus	Whole plant
	Inhibitors of the human protein tyrosine phosphatase 1B	NS
	5-Lipoxygenase inhibition and antiallergenic	Rhizomes

NS = Not specified

Table3 (Cont.). Medicinal properties of *Ardisia* species [5]

***Ardisia* species**	**Medicinal properties**	**Plant parts used**
Ardisia kusukuensis Hayata	Against *Mycobacterium tuberculosis* H37Rv	Whole plant
Ardisia maculosa Mez.	Antibiotic	Whole plant
	Heptoprotector	NS
Ardisia mamillata Hance	Respiratory tract infection and menstrual disorder	Roots
Ardisia pusilla A.DC.	Immunological function and anti-tumor activity	NS
Ardisia sieboldii Miq.	Hepatoprotector	NS
	5-Lipoxygenase inhibition	Bark
Ardisia silvestris Pit	Colic and stomachache	Branches and leaves
Ardisia solanacea Roxb.	To treat asthma	Leaves and flowers
	Febrifuge	Root bark
Ardisia squamulosa Presl	Antiviral against herpes simplex and adenoviruses	Stem and leaves
Ardisia teysmanniana Scheff.	Antimicrobial	Leaves
Ardisia villosa Roxb.	Analgesic	Whole plant
	Febrifuge and antitussive	Roots

NS = Not specified

Table 4. Phytochemical constituents of *Ardisia* species and plant parts used for isolation of compounds [3]

***Ardisia* species**	**Phytochemical constituents**	**Plant parts used**
Ardisia arborescens Wall. ex A. DC	Ardisianones A-E	NS
Ardisia colorata Roxb.	Bergenin; norbergenin; demehoxybergenin; alkylresorcinols; ardisiaphenols A-C; and embelin	Fruits
Ardisia compressa Kunth.	Ardisin	Leaves
Ardisia cornudentata Mez.	Ardisianone and cornudentanone	Whole plant
Ardisia crenata Sims.	Rapanone and embelin	Roots
	2-Hydroxy-5 methoxy-3-pentadecenyl-(tridecenyl-, tridecyl-)benzoquinone	Fruits
	Bergenin; friedelin; β-sitosterol and rapanone	Roots
	FR900359	Whole plant
	Cyclameritin A	Roots
	3 β-O-α-l-rhamnopyrasonyl-(1→4)-β-D-glucopyranosyl 1-(1→2)-[β-L-glucopyrasenosyl-(1→4)] α-L-arabinopyranoside	
	3β-O{α-l-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-dglucopyranosyl-(1→4)}α-l-arabinopyranoside}-16α-hydroxy13,28-epoxyolean-29-oic acid; and	
	3 β-O{α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-dglucopyranosyl-(1→4)]α-l-arabinopyranoside}-16α-hydroxy-13,28-epoxy-30,30-dimethoxyoleane	

NS = Not specified

Table 4 (Cont.). Phytochemical constituents of *Ardisia* species and plant parts used for isolation of compounds [3]

Ardisia species	Phytochemical constituents	Plant parts used
Ardisia crenata Sims.	Ardisiacrenosides A and B; and ardisiacrespins A and B	Roots
	Bergenin; 11-O-vanilloyl- and 11-O-(3,4-dimethylgalloyl)-bergenins;11-O-galloylbergenin and 11-O-syringylbergenin	Roots
Ardisia crispa (Thunb.) A.DC.	Ardisiacrispin A and B	Roots
	AC7-1	NS
Ardisia elliptica Thunb.	Bauerenol; and α- and β-amyrin	Leaves
	Bergenin	NS
Ardisia gigantifolia Stapf.	5-(Z-Nonadec-14-enyl) resorcinol	Roots
Ardisia iwahigensis Elmer	Ardisenone	Leaves & twigs
Ardisia japonica (Thunb.) Blume	Embelin; rapanone; and 2-hydroxy-5 methoxy-3-pentadecenyl-(tridecenyl-, tridecyl-)benzoquinone	Creeping rhizomes
	Ardisin	NS
	Ardisianones A and B; maesanin; and alkenyl 1-4 benzoquinone	Roots
	Bergenin and norbergenin ardisianones A and B	Aerial parts
Ardisia mamillata Hance	Ardisiamamillosides A and B	Roots
Ardisia macrocarpa Wall.	Rapanone	Bark & heartwood

NS = Not specified

Embelin

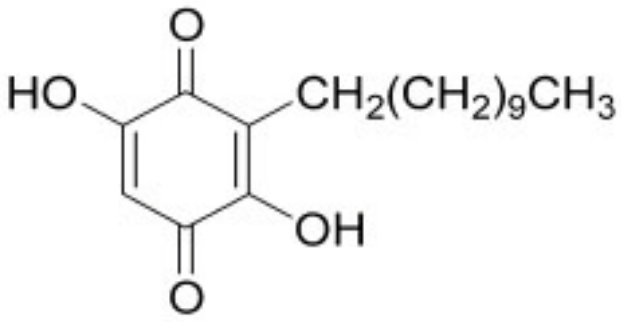


Figure 2. Structure of embelin

Scientific Name: 2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-benzoquinone

Molecular weight: 294.39

Description: Glistening orange plates from alcohol, benzene or acetic acid

Melting point: 142-143 °C.

Solubility: soluble in the usual hot organic solvents or in alkali hydroxide solutions, very slightly soluble in petroleum ether, practically insoluble in water [8].

Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) is a naturally occurring simple alkyl benzoquinone. It can be isolated from *Embelia ribes* Burm.f. and other species of Myrsinaceae family. This bright orange pigment is abundant in the family Myrsinaceae [9].

It has been reported many biological activities such as antibacterial [10], anticancer [11], anticonvulsant [12], and anti-mitotic [13], antidiabetic [14] activities, growth inhibition of HepG2 cell [15] and antifertility on male rats [16]. Embelin was found to reduce the total counts of *Heligmosomoides polygyrus* in mice significantly [17].

***Ardisia elliptica* Thunb.**

Ardisia elliptica Thunb., named PHI-LANG-KA-SA in Thai, has been reported many constituents such as embelin (major component), stigmasterol, stigmasteryl-3-*O*-palmitate, β-sitosteryl-3-*O*-plamitate, α-amyrin and others [4].

The dried fruit of this species is one of herbal crude drug traditionally used in Thailand for treatment of fever, diarrhea, allergy and imbalance of Dhatu. It is an ingredient in Thai traditional medicine recipe published in the List of Herbal Medicinal Products A.D.2011 namely, Apaisali (for treatment of flatulence), Plookfiretad (for galactagogue and postpartum tonic) and Prabchomphuthaveeb (for treatment of cold and allergic rhinitis) [18].

Phadungkit and Luanratana (2006) isolated syringic acid, isorhamnetin and quercetin from the ripe fruits of *A. elliptica* and reported the antibacterial activities against *Salmonella enteritidis, S. weltevreden, S. typhimurium* and *S. blockley* [4].

Embelin was found as the major component and exhibited the promising effects on cytotoxic activity against brine shrimp, DPPH scavenging, moderate effect on insect antifeedant activity, antimicrobial activity against *Bacillus subtilis* as well as enzymatic inhibition on p-hydroxypyruvate dioxygenase. A mixture of 1,3-dihydroxy-5-(heptadec-8,11-dienyl)benzene and 1,3-dihydroxy-5-(pentadec-8-enyl)benzene displayed strongly cytotoxic activity against the breast cancer cell and the small cell lung cancer [4,19].

Sumino and the colleages (2001, 2002) isolated ardisiphenols from the dried fruits and showed moderate DPPH scavenging activity and cytotoxicity against the murine breast cancer cell line, FM3A [20].

However, *A. elliptica* dried fruit crude drug monograph has not been published.

Quality control methods for herbal materials

According to WHO guidelines, plant materials have emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. This guideline has described detailed technical

information on various analytical methods for determining possible contaminants and residues in herbal medicines [1].

The chemical composition and bioactive compounds may vary dramatically with difference collection seasons and regions. Therefore, it is necessary to chemically standardize the herbal extracts or products for biological, pharmacological and clinical studies [21].

Macroscopic and Microscopic evaluation

An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of such materials, and should be carried out before any further tests are undertaken. Macroscopic identity of herbal materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface [1].

Determination of water content

An excess of water in herbal materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis.

The *azeotropic method* gives a direct measurement of the water present in the material being examined. The sample is distilled together with a water immiscible solvent, such as toluene or xylene, the water and the solvent are distilled together and separated in the receiving tube on cooling. It must be concerned that the solvent can absorb some water leading to false results. It is necessary to saturate the solvent with water before use [1].

Determination of Loss on drying

The test for *loss on drying* determines both water and volatile matter. Drying can be carried out either by heating to 100–105 °C or in a desiccator. The desiccation method is especially useful for materials that melt to a sticky mass at elevated temperatures [1].

Determination of ash and acid insoluble ash

The total ash method is designed to measure the total amount of material remaining after incineration. This includes both “physiological ash”, which is derived

from the plant tissue itself, and “non-physiological” ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface [1].

Acid-insoluble ash is the residue obtained after boiling the total ash with about 2 N hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth [1].

Determination of volatile oil

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. Chemically, they are usually composed of mixtures of, for example, monoterpenes, sesquiterpenes and their oxygenated derivatives. Aromatic compounds predominate in certain volatile oils. Because they are considered to be the “essence” of the herbal material, and are often biologically active, they are also known as “essential oils”. The term “volatile oil” is preferred because it is more specific and describes the physical properties [1].

Thin-layer chromatography

TLC is an easy economic and quick method that can be used for qualitative and quantitative analysis as well as purification of natural product. Practically, it is useful in identifying a known compound and detecting the presence of various types of secondary metabolites in herbs [21].

Thin-layer chromatography is often used as a qualitative method. It can determine the number of components in a mixture; verify the identity of substances [22].

As Hoeltz and colleagues explained that: [23]

“TLC is a simple and robust technique, which is relatively inexpensive to establish in a testing laboratory, and most laboratories in developing countries have considerable expertise and experience with it [23].”

The commonly sorbent used as solid phase for TLC plates include silica, alumina, octadecasilica (ODS), cellulose, dextran gels, polyamide or other ion exchange polymeric resin [21].

An important qualitative parameter in TLC of particular sorbent and solvent system is the Rf value. It is define as:

$$Rf = \frac{\text{Distance of compound from origin spot to the developed spot}}{\text{Distance of solvent from origin spot to the developed spot}}$$

For analytical TLC, effective detection is requires. Most of compounds are colorless under regular sunlight. Thus, they must be detected under other conditions. Detection could be detected by under UV light or color reaction with a reagent. Most UV lamps have two wavelengths of UV light, 254 nm and 365 nm. For the compounds that require spray detection. The mechanism of spray detection is the color reaction between the compound of interest on the TLC and the spray reagent. Some color reactions on TLC plates may need heating. The most popular spray reagents are listed in Table 5 [24-27].

TLC scanning had been widely applied in separation, identification and quantitative analysis of compounds in herbal extracts. In this method, TLC-densitometry is commonly used to scanning TLC plate. The signal of each substance zone is compared to the substance free plate background. For calibration and result calculation the obtained peak data of the unknowns are compared against data obtained for standards on the same plate. Quantitative evaluation can be performed with data from classical densitometry or with those from electronic image acquisition. Classical densitometry uses monochromatic light and a slit of selectable length and width to scan the tracks of a chromatogram, measuring the diffusely reflected light [28].

Figure 3. TLC-densitometry

Table 5. The most popular spray reagents [24-27]

Spray reagent	Recipe	Treatment	detection
p-Anisaldehyde / sulfuric acid	0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml conc. sulfuric acid.	Heat to 105 °C until maximum visualization of spots. Enhance background with water vapor spray. Components give violet, blue, red, grey or green spots.	Phenols, sugars, steroids, and terpenes.
Dragendorffs reagent.	Dissolve 0.11 g potassium iodide and 0.18 g bismuth subnitrate (OBiNO3) in 20 ml acetic acid and make up to 100ml.	Generally, color reaction occurs rapidly, but heat is required occasionally.	Alkaloids and quaternary nitrogen compounds
Ferric chloride	2.7 g of salt dissolved in 100ml 2M hydrochloric acid.		Phenols and phenolic acids.
Sulphuric acid spray reagent	5% w/v of the acid in ethanol.		Charring reagent for organic compounds. For detection of bile acids
Phosphomolydbic acid	0.25 g molybdatophosphoric acid in 50ml ethanol	Heat to 120 °C until spots appear If necessary; treat with ammonia vapors to remove some background coloration.	Reducing substances, e.g, alcohols, bile acids, lipids, fatty acids, steroids
Vanillin / sulfuric acid	Dissolve 1 g of vanillin in 100 ml conc.sulfuric acid. or 0.5 g vanillin in 80 ml sulfuric acid and 20 ml ethanol.	Heat at 120°C until maximum color development. Components give red and blue colors.	A universal spray, terpenoids, steroids saponins

High performance thin layer chromatography (HPTLC)

An HPTLC plate is generally applied for purification of compounds in a relatively simple fraction that have been separated by several chromatographic columns.

Analytical TLC and HPTLC plates are commercially available. They can also be prepared in the lab as well. Although it is cheap and fast, but the accuracy of the result cannot be compared with that obtained by HPLC or GC [21].

Quanlitative or semi-quantitative TLC analysis is usually done by visual comparison. Quantitative TLC analysis can be precisely performed by the technique of densitometry. It is based on measuring the absorbance or fluorescence of different zones on the plate exposed to monochromatic source of light. However, the densitometry equipment is also expensive [22].

In recent study, there have an alternative way to quantitative evaluation by the charged coupled devices, that are two-dimensional detectors containing an sensors that can imaging an area in a seconds or real time. The output from each sensor pixel on the CCD is a voltage, which is proportional to the intensity of light falling on the sensor and the exposure time. These series of voltages are digitized and transferred to a computer for storage and data processing. By coupling CCD detection with TLC, the entire chromatographic plate can be imaged in a single exposure yielding rapid quantification in shorter analysis time than of slit scanning densitometers. CCD detectors have demonstrated extremely low dark current and read noise characteristics, high sensitivity and excellent linearity. These features have made the CCD an excellent detector for many imaging applications in chemical analysis, such as fluorescence detection [23].

Figure 4. CCD camera and CCD detectors

Image processing

For image processing, there are many free programs have been developed in the last 50 years e.g. Scion Image, ImageJ. In the beginning ImageJ is being developed in the computer's mac because the graphics is better than PC. But the users have limits to their computer used. On the other hand, Scion image has been develops in the PC computer, to approach more users, unfortunately the Scion image is a close program that cannot solve the problem during the work and have more bugs that cannot be fix. In 2010 the ImageJ has been develops in Java programs, The package was developed and is maintained by Wayne Rasband, at the research services branch of the National Institute of Mental Health where is situated in Bethesda, Maryland in the USA. It is an open source that users or developer can help to fix the problem and develops the program to ultimate. This also free available in PC (windows) (http://rsbweb.nih.gov/ij/index.html) which can be afford to more users and very appropriate for the current application [29].

CHAPTER III

MATERIAL AND METHODOLOGY

Chemicals

Ammonia	BDH, Chemicals Ltd., England
Chloroform	J.T. Baker Chemical Co., Phillipsburg, USA
Embelin	ChromaDex, USA
Ethanol	RCI Labscan Limited, Bangkok, Thailand
Ethyl acetate	Mallinckrodt® Inc., USA
Formic acid	RCI Labscan Limited, Bangkok, Thailand
Hydrochloric acid	RCI Labscan Limited, Bangkok, Thailand
Methanol	RCI Labscan Limited, Bangkok, Thailand
N – butanol	RCI Labscan Limited, Bangkok, Thailand
N - propanol	BDH, Chemicals Ltd., England
Phosphomolybdic acid	SIGMA-ALDRICH, CO., St. Louis , USA
Toluene	RCI Labscan Limited, Bangkok, Thailand

The chemicals used were of analytical grade.

Materials

Filter paper No.4	Whatman^{TM} Papaer, UK
Filter paper No.40 ashless	Whatman^{TM} Papaer, UK
TLC silica 60F $_{254}$	MERCK LTD., USA

Instruments

HPTLC densitometer	US. CAMAG scientific, USA
Canon, PowerShot A650 IS camera	Canon Merketing (Thailand) Co.,LTD, Bangkok
ImageJ software	The National Institute of Mental Health, USA

Plant materials

The dried *Ardisia elliptica* fruits were purchased from 15 traditional drug stores at different locations throughout Thailand and authenticated by one of the authors (N.R.). Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University. The crude drug samples were examined and the other parts of plants, for example leaves, branches were removed. The crude drugs were kept in the closed container protected from light and heat.

Methods

Macroscopic evaluation

For macroscopic evaluation, the dried *Ardisia elliptica* fruits were identified for their sensory or organoleptic characters such as color, odor, taste, size, shape and other characters.

Microscopic evaluation

The microscopic appearances of the *Ardisia elliptica* fruits were determined in cross sectional view for anatomical characters and in powder form for histological characters. The section was performed by razor blade and the powders were sifted through a 250 micron sieve. These tissues in water were mounted onto a slide then inspected under microscope with a magnification of 4x, 10x and 40x. Photographs were taken with the help by a camera. The microscopic characters were drawn in the proportion size related to the original.

Determination of water content (Azeotropic method)

Weighed 50.00 g of the powdered dried *Ardisia elliptica* fruits and transferred to the flask. Added 600 ml of water saturated toluene and boiled the flask until the water was completely distilled, removed heat, allowed the receiving tube to cooled in room temperature and dislodged any droplets of water adhered to the walls of the receiving tube. Allowed the water and toluene layers to be separated and read off the volume of water, calculated the content of water as a percentage of dried material.

Determination of loss on drying

Placed 3.000 g of the powdered dried *Ardisia elliptica* fruits in a previously dried and tared crucible, dried the sample at 105°C for 6 hour and weighed, calculated the loss of weight in a percentage of dried material.

Determination of total ash

Placed 3.000 g of the powdered dried *Ardisia elliptica* fruits in a previously dried and tared crucible, ignited it by gradually increasing the heat to 600 °C until white, cooled in desiccator and weighed, calculated the content of total ash in a percentage of dried material.

Determination of acid insoluble ash

To the crucible containing the total ash, added 25.0 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled gently for 5 minutes. Rinsed the watch-glass with 5.0 ml of hot water and added this liquid to the crucible. Collected the insoluble matter on an ashless filter-paper No.40 and washed with hot water until the filtrate was neutral, transferred the filter-paper containing the insoluble matter to the original crucible, dried on a hot-plate and ignited to constant weight. Allowed the residue to cool in desiccator then weighed without delay, calculated the content of acid-insoluble ash in a percentage of dried material.

Determination of ethanol soluble extractive value

Placed 4.000 g of the powdered dried *Ardisia elliptica* fruits in a glass-stopper conical flask, macerated with 70.0 ml of 95% ethanol for 6 hours, frequently shaked then allowed standing for 18 hours. Filtered, washed the marc with ethanol and adjusted the filtrate to 100.0 ml with ethanol, transferred 25.0 ml of the filtrated to a tared beaker and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay, calculated the content of extractable matter in a percentage of dried materials.

Determination of water soluble extractive value

Placed 4.000 g of the powdered dried *Ardisia elliptica* fruits in a glass-stopper conical flask, macerated with 70.0 ml of water for 6 hours, frequently shaked then allowed standing for 18 hours. Filtered, washed the marc with water and adjusted the filtrate to 100.0 ml with ethanol, transferred 25.0 ml of the filtrated to a tared beaker and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours, cooled in desiccator for 30 minutes and weighed without delay, calculated the content of extractable matter in a percentage of dried materials.

Determination of volatile oil

Weighed 100.00 g of the powered dried *Ardisia elliptica* fruits, added 600.00 ml of water and distilled by Clevenger apparatus until the volatile oil completely distilled, allowed to cooled in room temperature then read out the volume of volatile oil, calculated the volatile oil content in a percentage of dried materials.

Thin layer chromatographic fingerprinting

From aforementioned ethanol soluble extractive procedure, dissolved the crude extract in methanol to 1 mg/ml. Applied 3.0 µl of ethanolic extract of *Ardisia elliptica* fruits on the silica gel 60 F_{254} TLC plate of 0.2 mm thickness, developed over a path of 10.0 cm using a mixture of chloroform, ethylacetate and formic acid (5:4:1). After development, the plate was visualized under UV 254 nm, 365 nm and phosphomolypbic acid staining.

Preparation of standard solutions

The stock solution of standard embelin (1 mg/ml) was prepared in methanol. The stock solution was appropriately diluted to obtain the series of standard solutions of concentration 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml. These solutions were stored in refrigerator at 4^{o}C.

Preparation of hexane extracts of *Ardisia elliptica* fruits

The powdered dried *Ardisia elliptica* fruits (20.0 g) were exhaustively extracted with hexane by soxhlet apparatus. The extract was filtered and evaporated until dryness under reduced pressure at≦ 50ºC. The yield was recorded. The extract was dissolved in methanol to get the concentration of 1.0 mg/ml. This extract was further used for TLC image analysis.

TLC image analysis of embelin

Applied 3.0 µl of hexane extract of *Ardisia elliptica* fruits and 3.0 µl of standard set of embelin solutions on the silica gel 60 F_{254} TLC plate of 0.2 mm thickness, developed over a path of 10.0 cm using a mixture of n-butanol, n-propanol and 4 N ammonia (1:7:2). After development, the plate was visualized under UV 254 nm.

The photos were taken using Canon, PowerShot A650 IS camera and stored as JPEG files with C mode ISO 80, fluorescent, largest and superfine image. The ImageJ software was used to analyze and quantitate the embelin spot on TLC plate. The calibration curve of embelin was prepared by plotting peak areas *vs.* concentrations of embelin applied.

TLC-densitometry of embelin

The TLC plates were scanned using densitometer (CAMAG, USA) to quantitatively analyze the TLC spots. The calibration curve of embelin was prepared by plotting peak areas *vs.* concentrations of embelin applied.

Method validation

The method is validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness according to the ICH guidelines [30].

Linearity

The calibration curve was obtained and the linearity range was evaluated.

Accuracy

The accuracy of the method was tested as the recovery of spiked known levels of standard embelin into the sample. Standard embelin solution was added at three different levels (0.15, 0.75, 1.35 µg/spot). At each level, three determinations were performed and the results were calculated by the amount difference between the spiked and un-spiked sample analyzed under the same conditions. The accuracy was determined by using following formulae:

% Recovery = $(C/(A+C_0)) \times 100$

C = Tested amount in recovery sample

A = Amount spiked into C_0

C_0 = Calculated theoretical pre-existing amount (determined from unspiked sample evaluation)

Precision

The precision of the method was verified by repeatability (intra-day) and intermediate precision (inter-day) studies. Intra-day and inter-day precision were perform by analyzing sample solution containing the embelin of 0.9, 2.1, 3 µg/µl three times on the same day and three different days respectively. The embelin content was calculated the coefficient of variation was expressed in term of % relative standard deviation (% RSD) by following formulae.

%RSD = SD x 100/Mean

Where, SD = the standard deviation of each measurement

Limit of detection

The limit of detection (LOD) was determined from the calibration curve replicates using following formula.

LOD = 3.3(SD)/S

Where, SD = the y-intercept standard deviation of regression line.

S = the slope of regression line

Limit of quantitation

The limit of quantitation (LOQ) was determined from the calibration curve replicates using following formula.

LOQ = 10 (SD)/S

Where, SD = the y-intercept standard deviation of regression line.

S = the slope of regression line

Data analysis

The parameters due to standardization were expressed as grand mean ± pooled standard deviation (SD)

The embelin content between TLC image analysis and TLC-densitometry were compared by Wilcoxon Signed Ranks Test.

CHAPTER IV

RESULTS

Pharmacognostic specification of *Ardisia elliptica* Thunb.

Common Name	PHI-LANG-KA-SA
Other Names	THU-LANG-KA-SA, RAAM-YAI
English Name	Shoebutton ardisia
Scientific Name	*Ardisia elliptica* Thunb.
Synonyms	*Ardisia squamulosa* Presl., *Ardisia humilis* auct., non Vahl., *Ardisia polycephala* Wall., *Ardisia solanacea* Roxb.,
Family	MYRSINACEAE
Distribution	Throughout tropics
Used Part	Fruits
Ethnomedical Uses	Treatment of fever, diarrhea

Description of plant

Shrubs 1-2 m tall, glabrous. Branchlets angular, 3-4.5 mm in diam., glabrous, conspicuously black punctate-lineate, longitudinally ridged. Petiole marginate, 5-10 mm; leaf blade oblanceolate or obovate, 6-16 × 3-7 cm, subleathery, dull and densely punctate abaxially, especially along margin, base cuneate, margin revolute, entire, apex obtuse or acute; lateral veins 12-34 on each side of midrib, marginal vein present. Inflorescences axillary or subterminal on basally thickened lateral branches, subumbellate or umbellate. Flowers leathery, pink or white, 6-8 mm. Pedicel ca. 1-2 cm, minutely and densely white verruculose, densely punctate. Sepals broadly ovate, ca. 1 mm, densely black punctate, base rugose and subauriculate, margin subentire, scarious, minutely ciliate, apex rounded. Petals almost free, broadly ovate, densely punctate, glabrous, margin hyaline, scarious, entire, apex long attenuate. Stamens subequalling petals; anthers linear-lanceolate, punctate dorsally, longitudinally dehiscent, transversely septate-lobed, apex apiculate. Pistil as long as petals; ovary glabrous, pellucid punctate; ovules numerous, multiseriate. Fruit subglobose, red or purplish black, ca. 8 mm in diam., minutely punctate, fleshy [31-32].

Macroscopic

Whole plant

Figure 5. The whole plant of *Ardisia elliptica* Thunb.

Figure 6. ***Ardisia elliptica*** **Thunb.**

Crude drugs

Figure 7. Crude drug of dried *Ardisia elliptica* fruit

Microscopic

Anatomical character

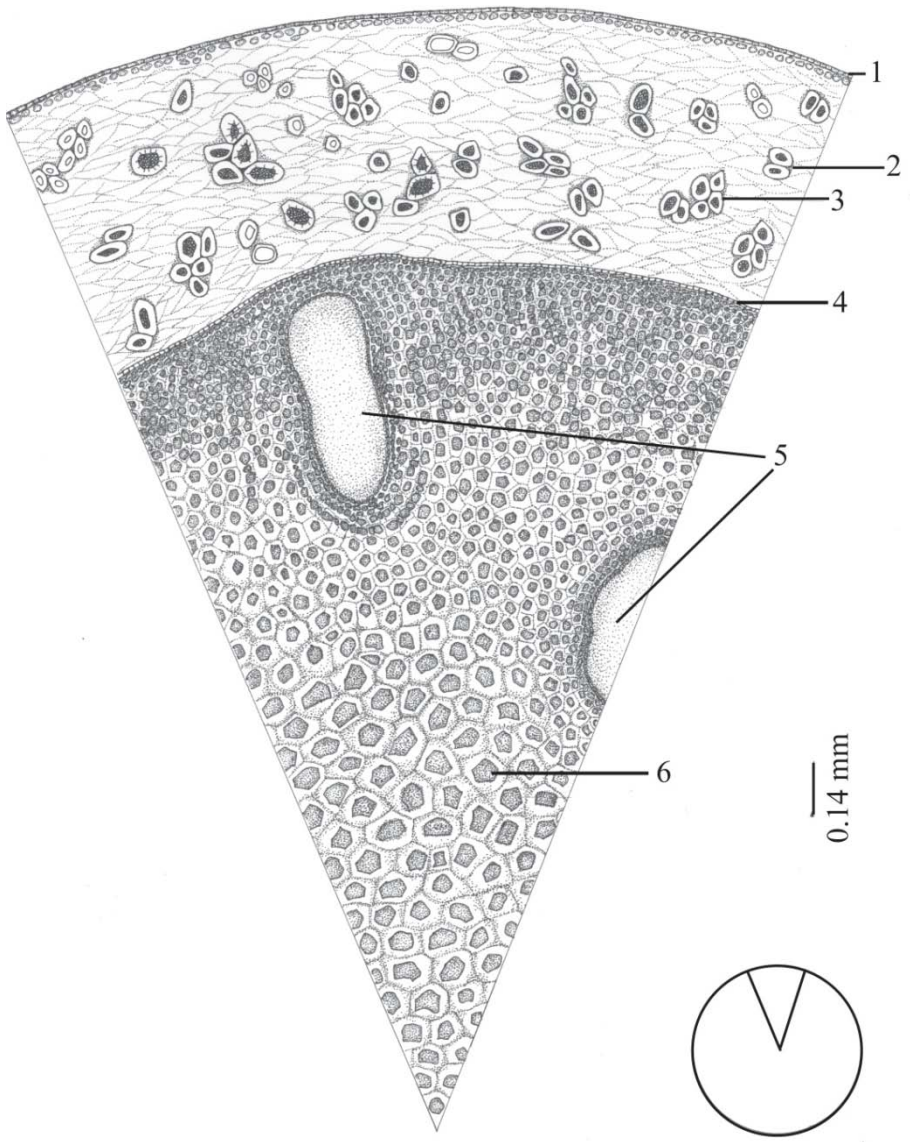


Figure 8: Transverse section of dried *Ardisia elliptica* fruit: 1. fruit wall, 2. mesocarp, 3. stone cell, 4. seed coat, 5. embryo sac, 6. endosperm

Microscopic

Histological character

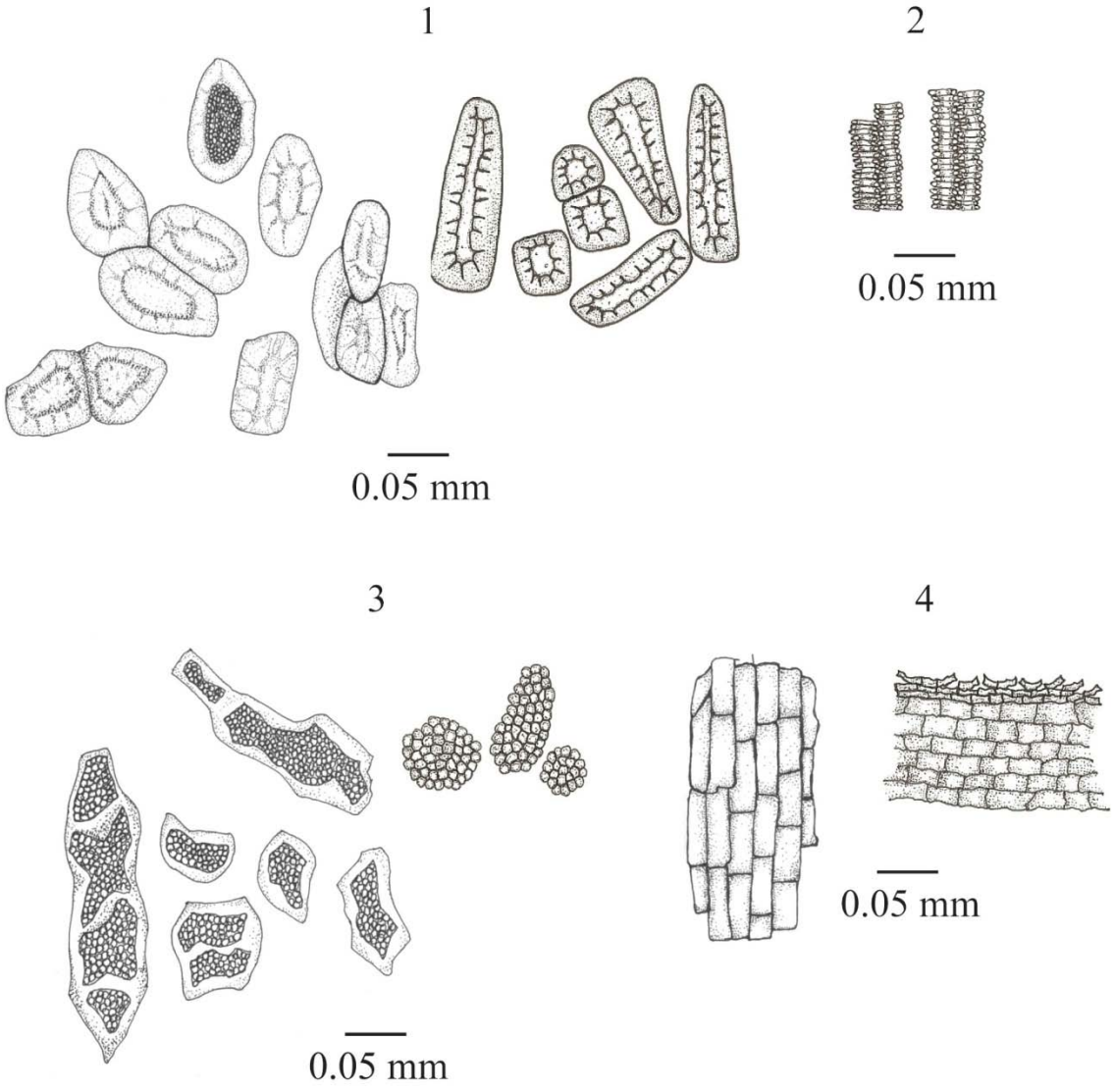


Figure 9: Powdered dried *Ardisia elliptica* fruit: 1. Sclereids or stone cell 2. Recticulated vessels 3. Endosperm 4. Epidermis of seed

Identification

TLC fingerprint

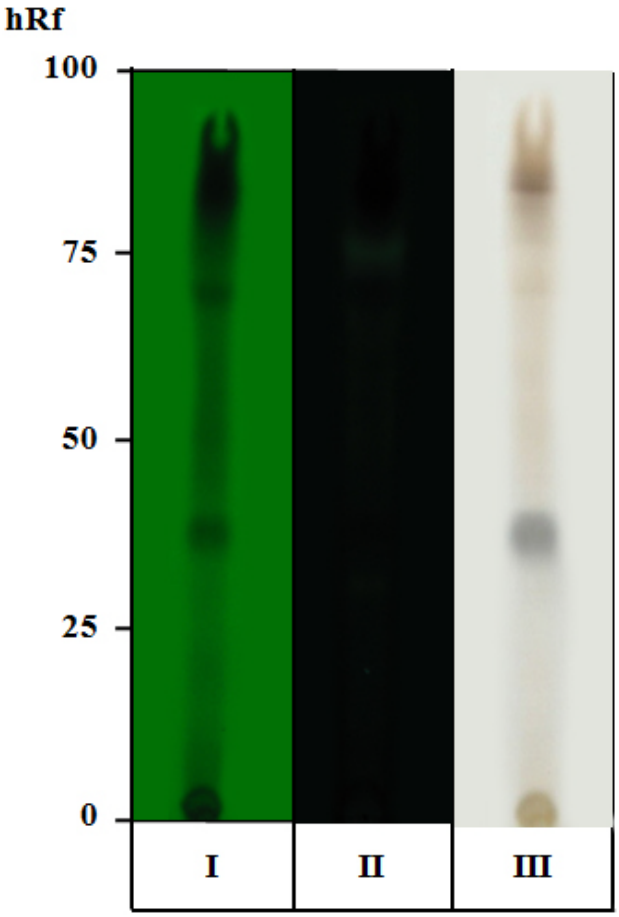


Figure 10: Thin-layer chromatogram of ethanolic extract of dried *Ardisia elliptica* fruit

Solvent system Chloroform: Ethyl acetate: Formic acid 5:4:1

Detection I = detection under UV light 254 nm

II = detection under UV light 365 nm

III = detection with Phosphomolybdic acid*, **

*Phosphomolybdic spray reagent

Preparation: 0.25 gm phosphomolybdic acid in 50 ml ethanol

**Spot color development

Heat to 120 ºC until spots appeared

Physico-chemical parameters

A pharmacognostic constant numbers due to the quality of dried *Ardisia elliptica* fruit were showed in table 6.

Table 6: Specification of dried *Ardisia elliptica* fruit.

Content (% by weight)	Mean	SD	Range (Mean ± 3SD)
Loss on drying	10.538	0.079	10.302 - 10.775
Total ash	5.526	0.152	5.071 - 5.980
Acid-insoluble ash	0.335	0.059	0.158 - 0.512
Ethanol-soluble extractive	4.916	0.446	3.579 - 6.252
Water-soluble extractive	9.418	0.910	6.688 - 12.148
Water content	10.613	0.689	8.546 - 12.681
Volatile oil content	0	0	0

* The parameters were shown as grand mean ± pooled SD. Samples were from 15 different sources throughout Thailand. Each sample was performed in triplicate.

Hexane extracts of dried *Ardisia elliptica* fruits

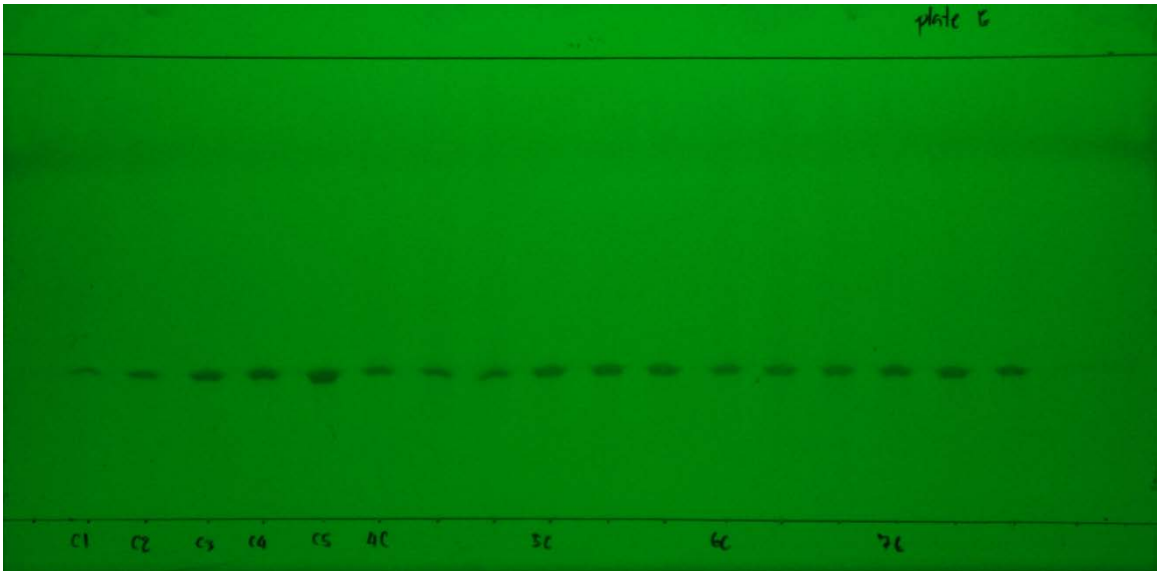
The percent yield of hexane extracts of dried *Ardisia elliptica* fruits were shown in table 7. The parameters were shown as a percentage of yield extract; each sample was done in triplicate.

Table 7. The percent yield of hexane extracts of dried *Ardisia elliptica* fruits (n=3)

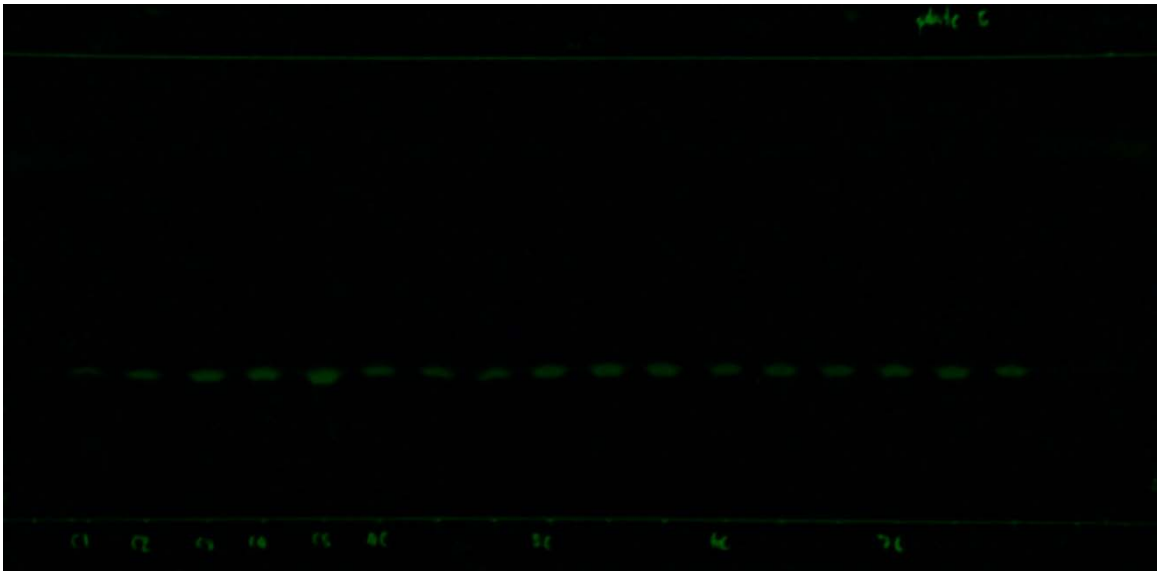
Source	weight of sample	weight of extractive value	% yield
1	20.0389	0.5991	2.99
2	20.0711	0.4565	2.27
3	20.0237	0.1264	0.63
4	20.0145	0.5009	2.50
5	20.0473	0.3191	1.59
6	20.0604	0.5047	2.52
7	20.0249	0.4749	2.37
8	20.0769	0.4427	2.21
9	20.0233	0.5698	2.85
10	20.0235	0.5835	2.91
11	20.0205	0.5910	2.95
12	20.0196	1.2165	6.08
13	20.0546	0.5820	2.90
14	20.0611	0.5592	2.79
15	20.0320	0.6208	3.10

TLC chromatogram of embelin

TLC chromatogram of embelin in dried *Ardisia elliptica* fruits were showed in figure 11. The quenching spot of embelin was obviously inspected and the background was clear. The retention factor (Rf) of embelin was around 35%.



(A)



(B)

Figure 11. The TLC plates developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background (B)

TLC densitometry

Calibration curve

The calibration curve of embelin by TLC densitomertry method was linear. The regression equations was y = 2602.2x + 638.34 and the correlation coefficients (r^2) were 0.9924. The linearity range of embelin was 0.60 - 3.00 µg (Figure 12).

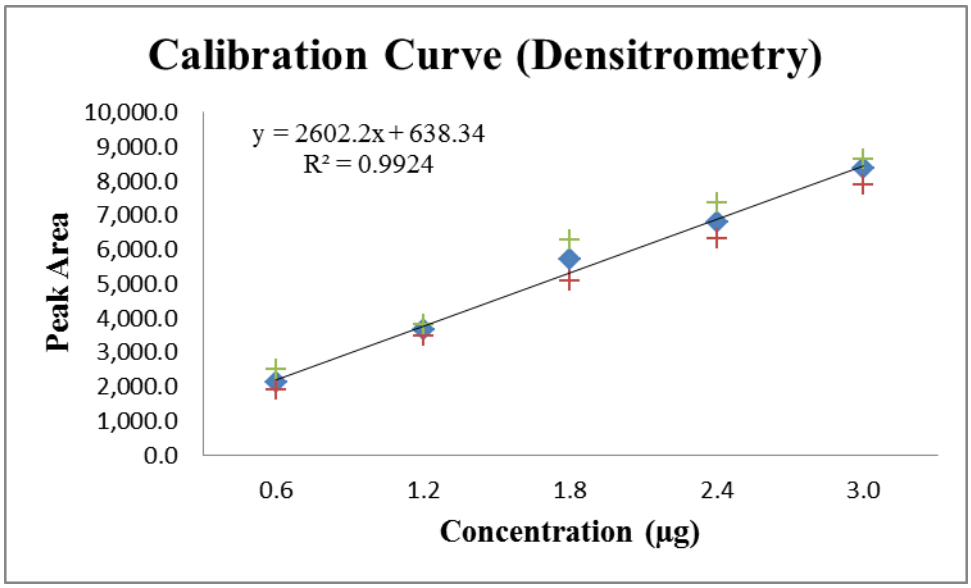


Figure 12. The calibration curve of Densitometry method

Accuracy

The accuracy of embelin quantitation by TLC densitomertry method was determined using recovery assay. The concentration of embelin in the sample before and after spiking with three levels of standard embelin (0.15, 0.75, 1.35 µg) were analyzed in triplicate. The values were shown as the proportion amount of tested embelin compared to the theoretical amount in percentage. The values were between 82.88 – 103.00 % recoveries as shown in table 8.

Table 8. Recovery of embelin (TLC-densitometry) (n=3)

Embelin added (µg)	Embelin found (µg)	% Recovery
0.00	1.23	-
0.15	1.42	103.00
0.75	1.97	99.67
1.35	2.13	82.88

Precision

The precision of embelin quantitation by TLC densitomertry method was determined in triplicates of each concentration group (0.90, 0.90, 3.00 µg). The values were shown as % Relative standard deviation which meant the error of the method. The Repeatability and intermediate precision were between 8.83 – 12.45 % RSD and 8.55 – 18.74 %RSD (table 9).

Table 9. Repeatability and intermediate precision of embelin (TLC-densitometry) (n=3)

Sample conc. (µg/spot)	Repeatability (% RSD)	Intermediate precision (% RSD)
0.90	12.45	13.63
2.10	8.83	8.55
3.00	11.40	18.74

Limit of Detection and Limit of Quantitation

In this study, limit of detection and limit of quantitation in TLC densitometry were based on the y-intercept standard deviation of the regression line. The residual standard deviation of the regression line was 250.25 and then calculated the y-intercept standard deviation of the regression line, the value was 16.59. the slope of the regression line was 2602.18. The LOD and LOQ for TLC densitometry were 21.04 and 63.76 ng respectively.

The amount of embelin in *Ardisia elliptica* fruit

The amounts of embelin in the hexane extracts were done in triplicate and evaluated by calibration curve. The values were calculated and shown as grams of embelin per 100 grams of dried fruits (table 10).

Table 10. The amount of embelin in *Ardisia elliptica* fruit in % by weight (TLC-densitometry) (n=3)

Source	Embelin in the hexane extract (µg/mg)		Yield of the hexane extract (g/100 g of dried fruit)	Embelin in *Ardisia elliptica* fruit (g/100 g of dried fruit)	
	Mean	SD		Mean	SD
1	0.3448	0.0356	2.9897	1.0308	0.1064
2	0.4429	0.0497	2.2744	1.0074	0.0867
3	0.3133	0.0052	0.6313	0.1978	0.1416
4	0.5742	0.0355	2.5027	1.4370	0.1131
5	0.6818	0.0195	1.5917	1.0852	0.4423
6	0.4911	0.0114	2.5159	1.2355	0.4717
7	0.5916	0.0696	2.3715	1.4031	0.0033
8	0.6985	0.0233	2.2050	1.5402	0.6716
9	0.4244	0.0138	2.8457	1.2076	0.6868
10	0.4081	0.0276	2.9141	1.1893	0.0888
11	0.4408	0.0110	2.9520	1.3013	0.2217
12	0.7458	0.0352	6.0765	4.5317	0.2496
13	0.9971	0.1315	2.9021	2.8936	0.0311
14	0.8972	0.0048	2.7875	2.5011	0.0795
15	0.6563	0.0209	3.0990	2.0339	0.1102

TLC image analysis by ImageJ software with CCD camera

Calibration curve

The calibration curve of embelin by TLC image analysis method was linear. The regression equations was y = 16288x + 3777.8 and the correlation coefficients (r^2) was 0.9936. The linearity range of embelin was 0.60 - 3.00 µg (Figure 13).

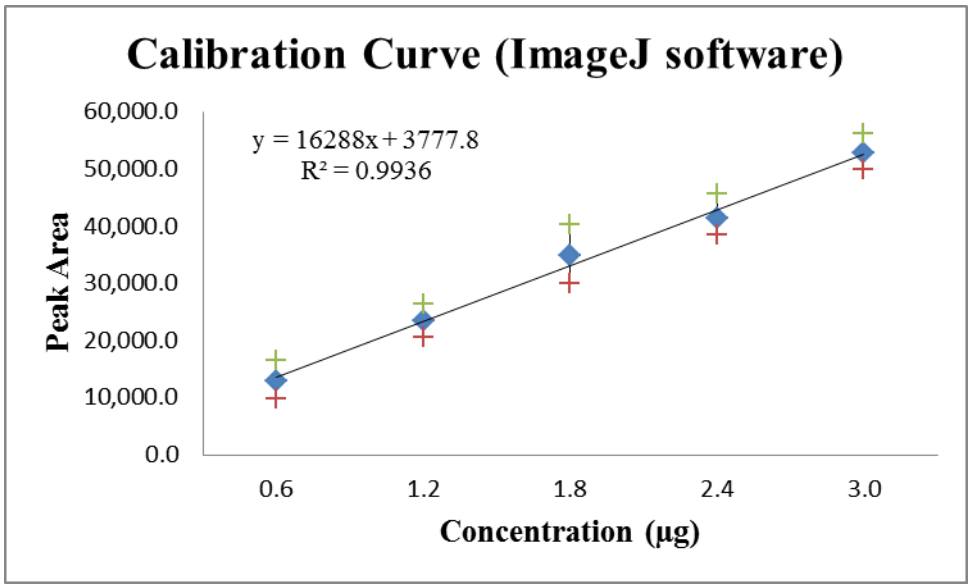


Figure 13. The calibration curve of TLC image analysis method

Accuracy

The accuracy of embelin quantitation by TLC image analysis method was determined using recovery assay. The concentration of embelin in the sample before and after spiking with three levels of standard embelin (0.15, 0.75, 1.35 µg) were analyzed in triplicate. The values were shown as the proportion amount of tested embelin compared to the theoretical amount in percentage. The values were between 103.83 – 123.50 % recoveries as shown in (table 11).

Table 11. Recovery of embelin (TLC image analysis) (n=3)

Embelin added (µg)	Embelin found (µg)	%recovery
0.00	1.02	-
0.15	1.22	103.83
0.75	2.15	121.49
1.35	2.93	123.50

Precision

The precision of embelin quantitation by TLC image analysis method was determined in triplicates of each concentration group (0.90, 0.90, 3.00 µg). The values were shown as % Relative standard deviation which meant the error of the method. The Repeatability and intermediate precision were between 5.27 – 8.12 % RSD and 1.92 – 9.29 %RSD (table 12).

Table 12. Repeatability and intermediate precision of embelin (TLC image analysis) (n=3)

Sample conc. (µg/spot)	Repeatability (% RSD)	Intermediate precision (% RSD)
0.9	5.99	1.92
2.1	5.27	9.29
3.0	8.12	6.53

Limit of Detection and Limit of Quantitation

In this study, Limit of detection and limit of quantitation in TLC image analysis were based on the y-intercept standard deviation of the regression line. The residual standard deviation of the regression line was 1436.69 and then calculated the y-intercept standard deviation of the regression line, the value was 39.75. the slope of the regression line was 16288.2. The LOD and LOQ for TLC image analysis were 8.05 and 24.40 ng respectively.

The amount of embelin in *Ardisia elliptica* fruit

The amounts of embelin in the hexane extracts were done in triplicate and evaluated by calibration curve. The values were calculated and shown as grams of embelin per 100 grams of dried fruits (table 13).

Table 13. The amount of embelin in *Ardisia elliptica* fruit in % by weight (TLC image analysis) (n=3)

Source	Embelin in the hexane extract (µg/mg)		Yield of the hexane extract (g/100 g of dried fruit)	Embelin in *Ardisia elliptica* fruit (g/100 g of dried fruit)	
	Mean	SD		Mean	SD
1	0.2615	0.0287	2.9897	0.7819	0.0858
2	0.4561	0.0267	2.2744	1.0374	0.1832
3	0.3613	0.0228	0.6313	0.2281	0.1073
4	0.4232	0.0119	2.5027	1.0590	0.0607
5	0.6170	0.0340	1.5917	0.9820	0.4466
6	0.4429	0.0358	2.5159	1.1142	0.4660
7	0.5601	0.0648	2.3715	1.3283	0.0144
8	0.6899	0.0818	2.2050	1.5211	0.4983
9	0.3097	0.0094	2.8457	0.8812	0.4888
10	0.3219	0.0154	2.9141	0.9380	0.0299
11	0.2944	0.0147	2.9520	0.8690	0.0386
12	0.7296	0.0233	6.0765	4.4335	0.0389
13	1.0565	0.1458	2.9021	3.0659	0.0541
14	0.9753	0.0607	2.7875	2.7188	0.0665
15	0.6846	0.0125	3.0990	2.1216	0.1461

Comparison of embelin contents between TLC densitometry and TLC image analysis

The comparison of embelin content between TLC densitometry and TLC image analysis (Table 14) were statistically tested using Wilcoxon Signed Ranks Test. It was found that the embelin contents by two methods were not significantly different (Z = -1.761, *P* = 0.078).

Table 14. The comparison of embelin content between TLC densitometry and TLC image analysis

Source	% Embelin content	
	TLC densitometry	TLC image analysis
1	1.0308	0.7819
2	1.0074	1.0374
3	0.1978	0.2281
4	1.4370	1.0590
5	1.0852	0.9820
6	1.2355	1.1142
7	1.4031	1.3283
8	1.5402	1.5211
9	1.2076	0.8812
10	1.1893	0.9380
11	1.3013	0.8690
12	4.5317	4.4335
13	2.8575	3.0730
14	2.5011	2.7188
15	2.0339	2.1216

CHAPTER V

DISSUSSION AND CONCLUSIONS

Quality of crude drugs is important for safety and efficacy of herbal medicine. It is necessary for the development of herbal remedy standardization for quality, safety and efficacy assurance in herbal medicine. In the present day, WHO launches the manual “Quality Control Methods for Medicinal Plant Materials” to be a guideline used for crude drug specification [1]. The usage and trend of the herbal medicine are increasing so the quality parameters can increase the confidence of practitioners and consumers and they are also important as the database for herbal medicine development.

In Thailand, the standardization of herbal remedies is needed. Traditional Thai medicine is composed of many herbal crude drugs. The pharmacognostic specification of each crude drug or plant material is concerned and the process is ongoing. This study provided the information of pharmacognostic specification and embelin content of *Ardisia elliptica* dried fruit which is one of herbal crude drug traditionally used in Apaisali, Plookfiretad, Prabchomphuthaveeb formulations [18].

The botanical specification in terms of anatomical and histological structures of plant materials are characteristics of each species and part used which can help in crude drug authentication [21].

The physico-chemical specification of the crude drug is another important parameter to represent the characteristics as well as the quality of crude drug as following:

Water content or moisture content indicates a physical factor accelerates crude drug deterioration. An excess of water in herbal materials will encourage microbial growth as well as plant decomposing enzyme activity. The moisture content should be not more than 13% for safe storage of plant materials [33]. The average moisture content of *Ardisia elliptica* dried fruit crude drug revealed in this study is around 10%. There is no volatile oil in this crude drug so the content of loss on drying is mainly from the water in the plant material. The ashes parameter is designed to measure the inorganic substances which remaining after incineration of crude drug.

The ashes can be from the plant tissue itself and the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. The acid insoluble ash indicates, the amount of silica presented from such as sand and siliceous earth [1]. The chemical parameter which evidences the apparent pharmacological substances is the extractive value by specified solvent. Ethanol and water were used in this study to extract a variety of compounds cover from non-polar to polar properties. The physico-chemical parameters provided by this study can be used for the quality control of *Ardisia elliptica* dried fruit crude drug.

Thin layer chromatogram can be used as crude drug fingerprint of chemical identification which supports crude drug authentication. This study demonstrated Chloroform: Ethyl acetate: Formic acid (5:4:1) as developing system. Visualization could be obtained by UV quenching at 254 nm or phosphomolybdic acid reagents.

For the development of embelin analysis, TLC is appropriate technique for compound separation and quantification. Several trials for the optimal mobile phase were done and the mixture of n-butanol, n-propanol, 4 N ammonia (1:7:2) was efficacious as previously reported [35]. The quenching spot of embelin was clearly seen. The Rf value was 0.35, as the same of our study [35].

The calibration curve of embelin by both TLC-densitometry and TLC image analysis–was linear. However detection principle between two methods is different, TLC-densitometry uses the detector to measure the light that emits from the sample but TLC image analysis used the CCD camera to photographing and collected the color in the area. The correlation coefficient (r^2) was more than 0.99 in range 0.60 – 3.00 µg/spot. This is good respond for linearity in herbal medicine.

Moreover, the accuracy between both methods was shown in a good result. This was meant the embelin can be measured by both methods. The accuracies of TLC-densitometry were between 82.88 – 103.00 % recoveries, TLC image analysis were between 103.83 – 123.50 % recoveries.

However, the precision between both methods was shown in different result. The TLC image analysis was shown better value than TLC-denstitometry. This might be affected by the different peak area respond operating program between both

methods because TLC-densitometry usually had auto-analyze the peak area but the TLC image analysis must do manual analyze by the users. The Repeatability and intermediate precision of TLC-densitometry were between 8.83 – 12.45 % RSD and 8.55 – 18.74 %RSD and TLC image analysis were between 5.27 – 8.12 % RSD and 1.92 – 9.29 %RSD.

Limit of Detection and Limit of Quantitation in both method were shown in the same value that are not much different. The LOD and LOQ for TLC densitometry were 21.04 and 63.76 ng respectively and TLC image analysis were 8.05 and 24.40 ng respectively.

For the amount of embelin content in 15 *Ardisia elliptcia* fruits difference places vender throughout Thailand, the results of TLC-densitometry were between 0.1978 - 4.5317 g/100g crude drug and TLC image analysis were between 0.2281 - 4.4335 g/100g crude drug (see table 14). According to the previous reported, in other species, *Embelia ribes* Brum., there were the embelin content around 3.813 g/100g [35]

The TLC densitometry and TLC image analysis were compared statistically tested using Wilcoxon Signed Ranks Test. It was found that the embelin contents by two methods were not significantly different ($Z = -1.761$, $P = 0.078$). Thus, the TLC image analysis is efficient way to use and suitable for the determination of substances by thin-layer chromatographic techniques which meant that the TLC image analysis is a method that is fast, easy and inexpensive to implement as the work that had been reported previously [22].

The benefits of the TLC image analysis are simple, quick, cheap, easy to modify, in each experiment, and can be applied in many other experiments [36].

Limitations of the TLC image analysis are if quenching spot are goes with a tail, it will cause distortion measurements and there are need to set up the proper light exposure in camera, if you set the exposure compensation wrong, the photos are distorted from their true light [23].

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APPENDIX

Table 15. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Bangkok 1

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.63		
	2	9.69		
	3	9.75	9.69	0.06
Total ash	1	4.58		
	2	4.80		
	3	4.72	4.70	0.11
Acid-insoluble ash	1	0.15		
	2	0.18		
	3	0.20	0.18	0.02
Ethanol-soluble extract~~ive~~	1	5.07		
	2	5.22		
	3	5.49	5.26	0.21
Water-soluble extract~~ive~~	1	10.38		
	2	9.78		
	3	9.56	9.90	0.43
Water content	1	8.00		
	2	9.49		
	3	9.19	8.89	0.79
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 16. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Bangkok 2

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	12.09		
	2	12.06		
	3	12.12	12.09	0.03
Total ash	1	5.09		
	2	4.83		
	3	5.08	5.00	0.15
Acid-insoluble ash	1	0.26		
	2	0.30		
	3	0.30	0.29	0.03
Ethanol-soluble extractive	1	8.30		
	2	8.22		
	3	9.29	8.60	0.59
Water-soluble extractive	1	15.31		
	2	13.00		
	3	13.36	13.89	1.24
Water content	1	10.59		
	2	11.18		
	3	11.39	11.05	0.41
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 17. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Bangkok 3

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.91		
	2	9.97		
	3	9.91	9.93	0.04
Total ash	1	5.48		
	2	5.33		
	3	5.63	5.48	0.15
Acid-insoluble ash	1	0.20		
	2	0.19		
	3	0.25	0.21	0.03
Ethanol-soluble extractive	1	6.31		
	2	6.00		
	3	5.67	5.99	0.32
Water-soluble extractive	1	10.13		
	2	10.07		
	3	12.40	10.87	1.33
Water content	1	10.59		
	2	9.99		
	3	10.59	10.39	0.35
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 18. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Buri Ram

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.22		
	2	10.19		
	3	10.24	10.22	0.02
Total ash	1	7.16		
	2	7.18		
	3	7.20	7.18	0.02
Acid-insoluble ash	1	0.33		
	2	0.34		
	3	0.30	0.32	0.02
Ethanol-soluble extractive	1	1.65		
	2	1.53		
	3	1.03	1.40	0.33
Water-soluble extractive	1	6.22		
	2	8.49		
	3	6.53	7.08	1.23
Water content	1	11.19		
	2	11.39		
	3	10.40	10.99	0.52
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 19. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Chiang Mai

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.21		
	2	9.24		
	3	9.30	9.25	0.04
Total ash	1	5.24		
	2	5.31		
	3	5.26	5.27	0.04
Acid-insoluble ash	1	0.43		
	2	0.31		
	3	0.17	0.30	0.13
Ethanol-soluble extractive	1	4.60		
	2	3.22		
	3	3.64	3.82	0.71
Water-soluble extractive	1	6.86		
	2	6.47		
	3	5.69	6.34	0.60
Water content	1	10.98		
	2	11.39		
	3	11.49	11.29	0.27
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 20. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Chumphon 1

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.54		
	2	10.54		
	3	10.58	10.55	0.02
Total ash	1	5.63		
	2	5.59		
	3	5.84	5.69	0.13
Acid-insoluble ash	1	0.28		
	2	0.25		
	3	0.26	0.26	0.01
Ethanol-soluble extractive	1	5.12		
	2	4.20		
	3	5.44	4.92	0.64
Water-soluble extractive	1	8.64		
	2	8.16		
	3	8.89	8.57	0.37
Water content	1	10.59		
	2	11.39		
	3	10.80	10.93	0.41
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 21. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Chumphon 2

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.80		
	2	9.77		
	3	9.78	9.78	0.01
Total ash	1	6.80		
	2	6.83		
	3	6.48	6.70	0.20
Acid-insoluble ash	1	0.65		
	2	0.61		
	3	0.52	0.59	0.07
Ethanol-soluble extractive	1	5.19		
	2	5.67		
	3	5.34	5.40	0.24
Water-soluble extractive	1	7.10		
	2	7.35		
	3	6.80	7.08	0.28
Water content	1	9.79		
	2	9.60		
	3	9.79	9.73	0.11
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 22. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Nakhon Nayok

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.74		
	2	10.68		
	3	10.61	10.68	0.06
Total ash	1	5.67		
	2	5.64		
	3	5.55	5.62	0.06
Acid-insoluble ash	1	0.46		
	2	0.41		
	3	0.52	0.47	0.05
Ethanol-soluble extractive	1	5.55		
	2	4.76		
	3	5.21	5.18	0.40
Water-soluble extractive	1	8.14		
	2	8.99		
	3	8.20	8.44	0.47
Water content	1	11.38		
	2	11.58		
	3	10.99	11.32	0.30
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 23. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Nakhon Pathom

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	11.34		
	2	11.36		
	3	11.42	11.37	0.04
Total ash	1	6.00		
	2	6.08		
	3	5.86	5.98	0.11
Acid-insoluble ash	1	0.29		
	2	0.37		
	3	0.26	0.31	0.06
Ethanol-soluble extractive	1	4.28		
	2	4.20		
	3	4.28	4.25	0.04
Water-soluble extractive	1	10.34		
	2	9.73		
	3	10.71	10.26	0.50
Water content	1	7.60		
	2	8.39		
	3	10.40	8.79	1.44
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 24. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Phetchabun

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.42		
	2	10.71		
	3	10.72	10.62	0.17
Total ash	1	4.97		
	2	4.90		
	3	4.99	4.96	0.05
Acid-insoluble ash	1	0.42		
	2	0.42		
	3	0.57	0.47	0.09
Ethanol-soluble extractive	1	4.21		
	2	5.24		
	3	5.12	4.86	0.57
Water-soluble extractive	1	8.29		
	2	8.15		
	3	9.49	8.64	0.74
Water content	1	11.58		
	2	10.39		
	3	11.00	10.99	0.59
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 25. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Ratchaburi

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.90		
	2	9.80		
	3	9.95	9.88	0.08
Total ash	1	5.79		
	2	5.24		
	3	6.01	5.68	0.40
Acid-insoluble ash	1	0.25		
	2	0.25		
	3	0.37	0.29	0.07
Ethanol-soluble extractive	1	3.77		
	2	3.84		
	3	3.00	3.54	0.46
Water-soluble extractive	1	6.06		
	2	5.91		
	3	6.50	6.16	0.31
Water content	1	11.33		
	2	11.45		
	3	10.46	11.08	0.54
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 26. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Rayong

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	9.93		
	2	9.99		
	3	9.71	9.88	0.15
Total ash	1	5.27		
	2	5.43		
	3	5.58	5.43	0.15
Acid-insoluble ash	1	0.28		
	2	0.33		
	3	0.41	0.34	0.06
Ethanol-soluble extractive	1	3.00		
	2	3.37		
	3	2.91	3.09	0.24
Water-soluble extractive	1	11.12		
	2	8.48		
	3	9.92	9.84	1.32
Water content	1	9.47		
	2	10.28		
	3	10.96	10.24	0.75
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 27. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Surat Thani

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.55		
	2	10.47		
	3	10.35	10.46	0.10
Total ash	1	5.15		
	2	5.21		
	3	4.94	5.10	0.14
Acid-insoluble ash	1	0.50		
	2	0.57		
	3	0.45	0.51	0.06
Ethanol-soluble extractive	1	4.07		
	2	3.40		
	3	3.74	3.73	0.33
Water-soluble extractive	1	6.76		
	2	6.75		
	3	7.62	7.05	0.50
Water content	1	13.19		
	2	12.49		
	3	10.49	12.06	1.40
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 28. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Ubon Ratchathani

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	10.95		
	2	10.94		
	3	10.94	10.95	0.00
Total ash	1	5.25		
	2	5.30		
	3	5.19	5.25	0.06
Acid-insoluble ash	1	0.23		
	2	0.27		
	3	0.19	0.23	0.04
Ethanol-soluble extractive	1	3.79		
	2	3.95		
	3	4.32	4.02	0.27
Water-soluble extractive	1	11.28		
	2	12.23		
	3	10.82	11.44	0.72
Water content	1	10.19		
	2	10.19		
	3	9.98	10.12	0.12
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

Table 29. Quality parameters (% by weight) of *Ardisia elliptica* fruits form Uthai Thani

Parameter	Crude drug sample no.	Amount (% by weight)	Mean	SD
Loss on drying	1	12.76		
	2	12.82		
	3	12.60	12.73	0.11
Total ash	1	4.90		
	2	4.89		
	3	4.75	4.85	0.08
Acid-insoluble ash	1	0.25		
	2	0.28		
	3	0.23	0.26	0.02
Ethanol-soluble extractive	1	10.22		
	2	9.89		
	3	8.91	9.68	0.68
Water-soluble extractive	1	14.32		
	2	15.10		
	3	17.74	15.72	1.79
Water content	1	11.99		
	2	11.00		
	3	11.00	11.33	0.57
Volatile oil content	1	0.00		
	2	0.00		
	3	0.00	0.00	0.00

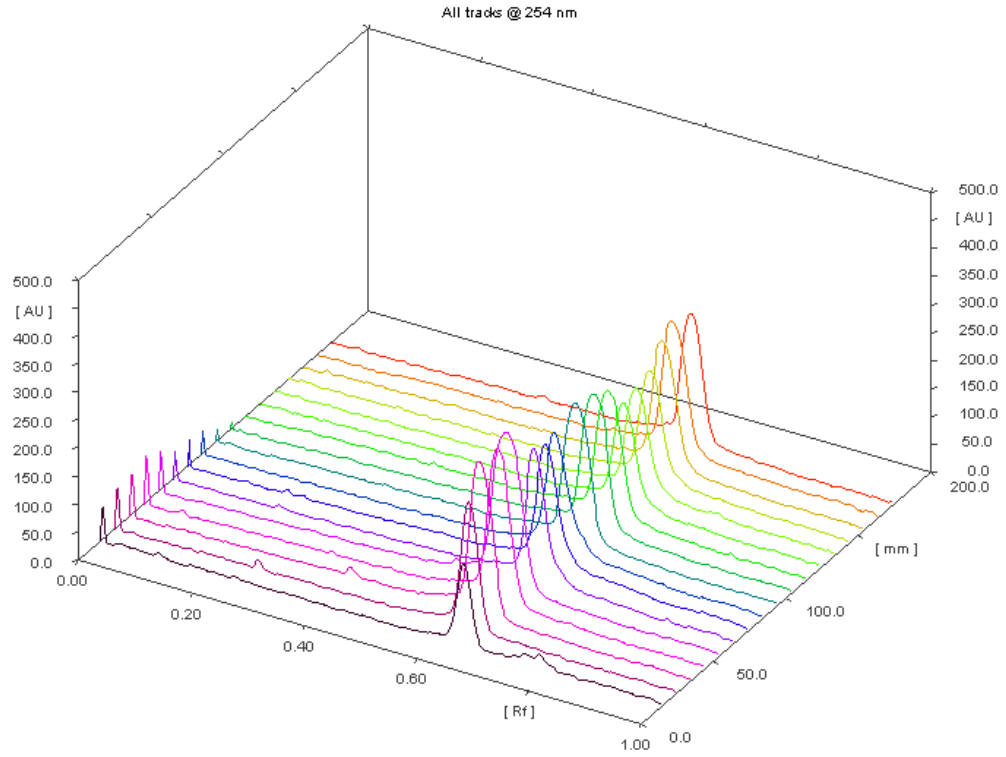


Figure 14. 3D TLC-densitometry chromatogram (plate A–Accuracy)

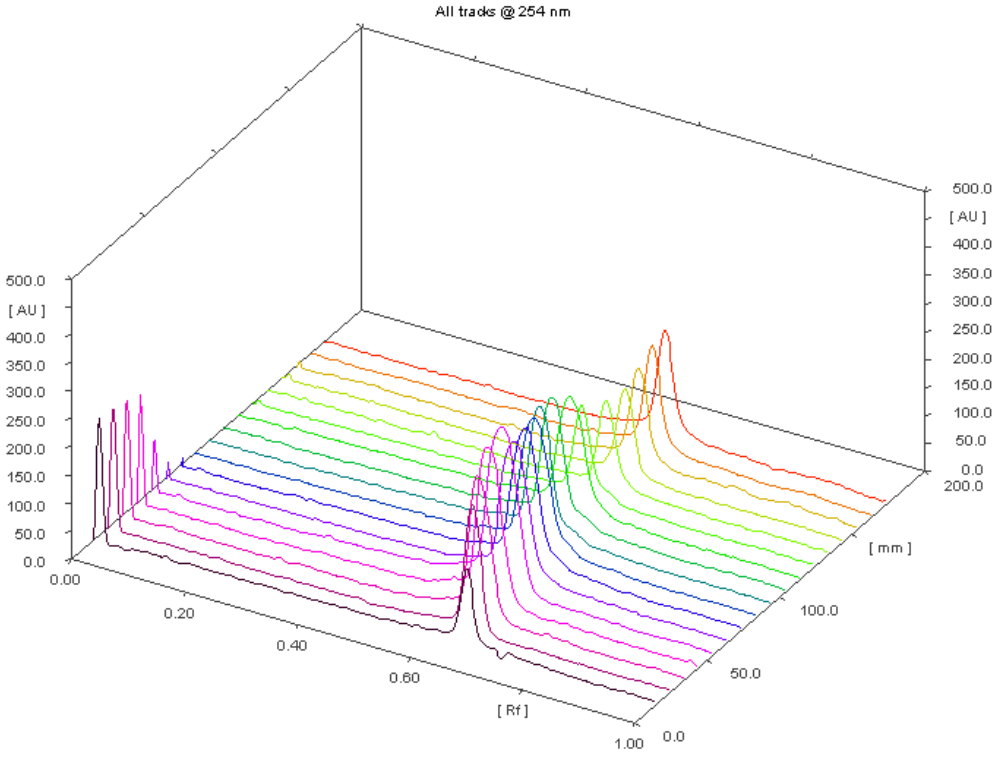


Figure 15. 3D TLC-densitometry chromatogram (plate B –Precision & sample No.1)

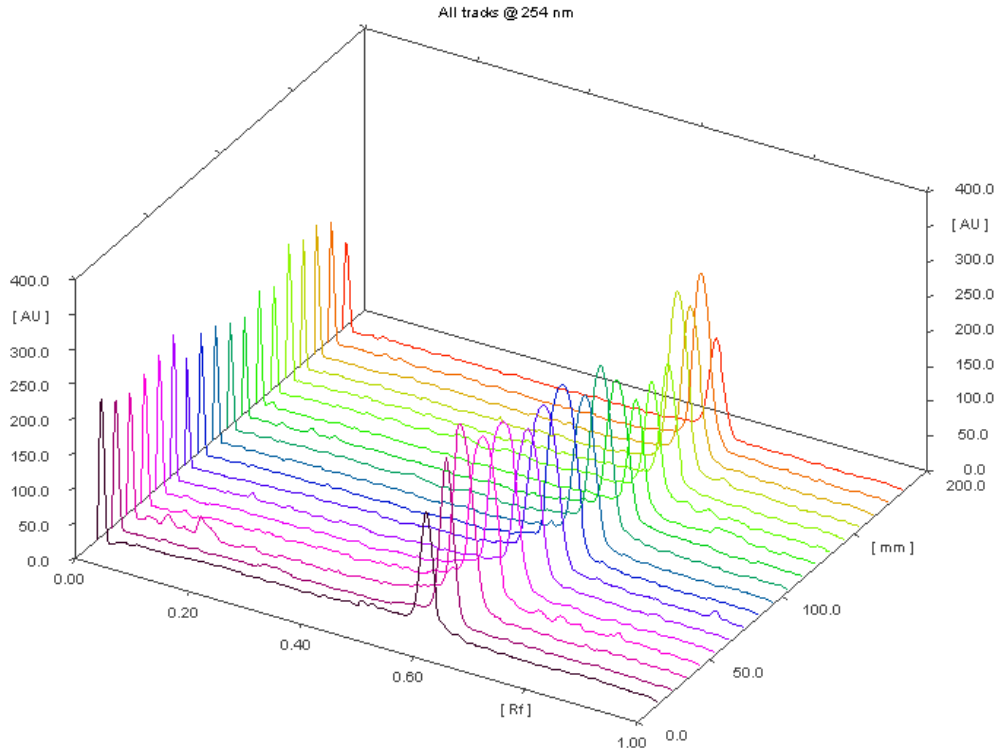


Figure 16. 3D TLC-densitometry chromatogram (plate C – Precision & sample No.2)

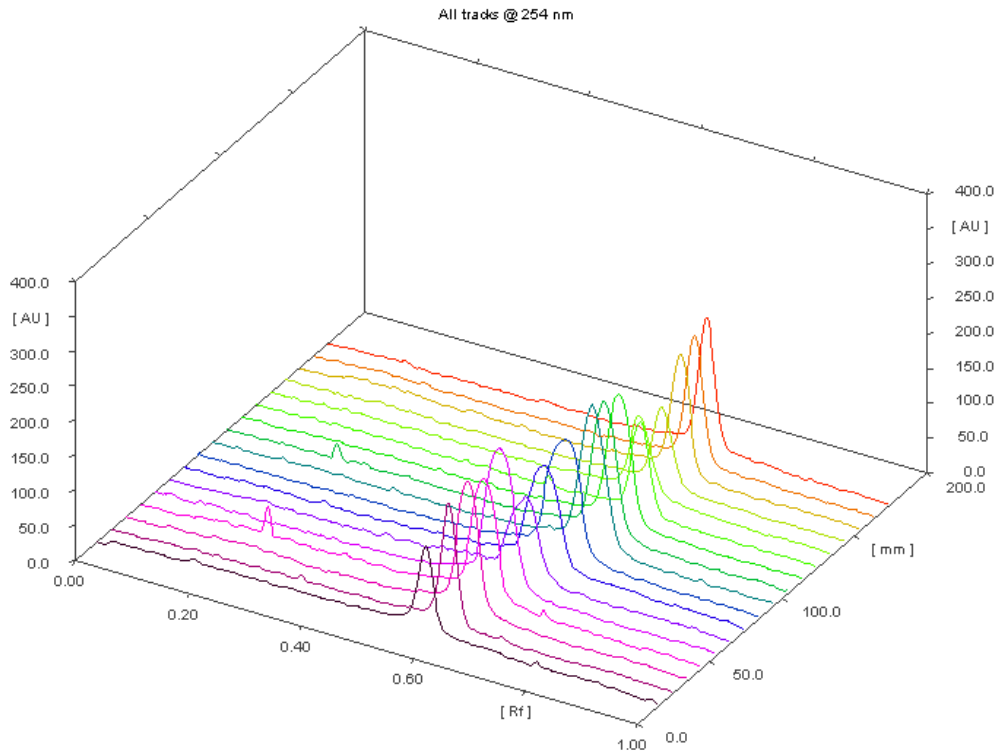


Figure 17. 3D TLC-densitometry chromatogram (plate D – Precision & sample No.3)

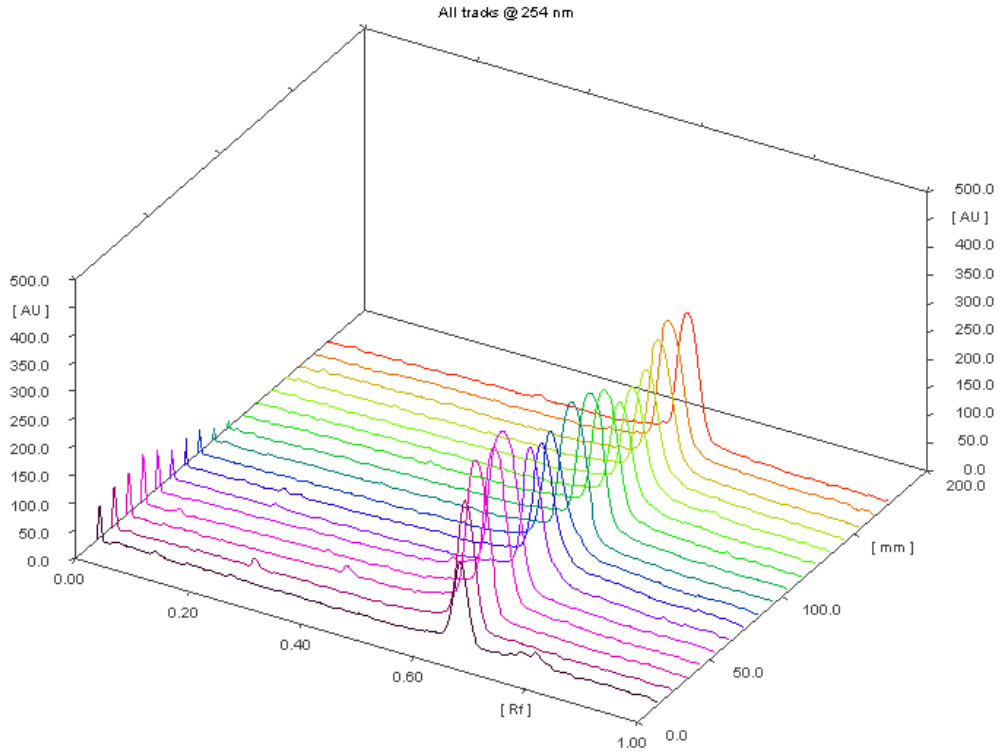


Figure 18. 3D TLC-densitometry chromatogram (plate E – sample No. 4-7)

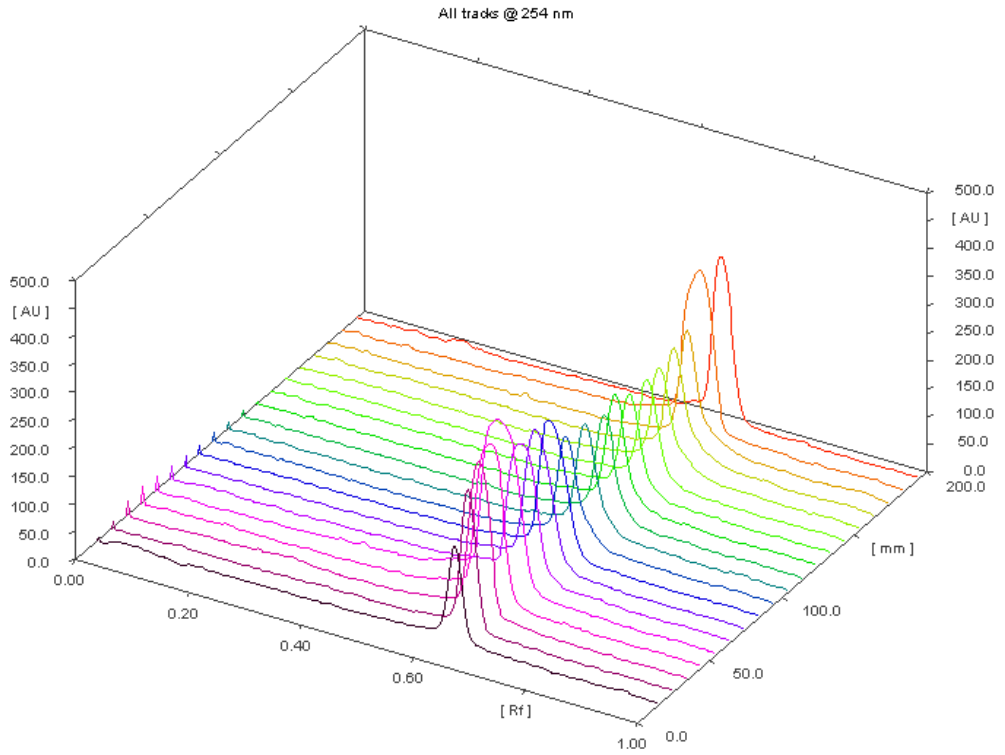


Figure 19. 3D TLC-densitometry chromatogram (plate F – sample No. 8-11)

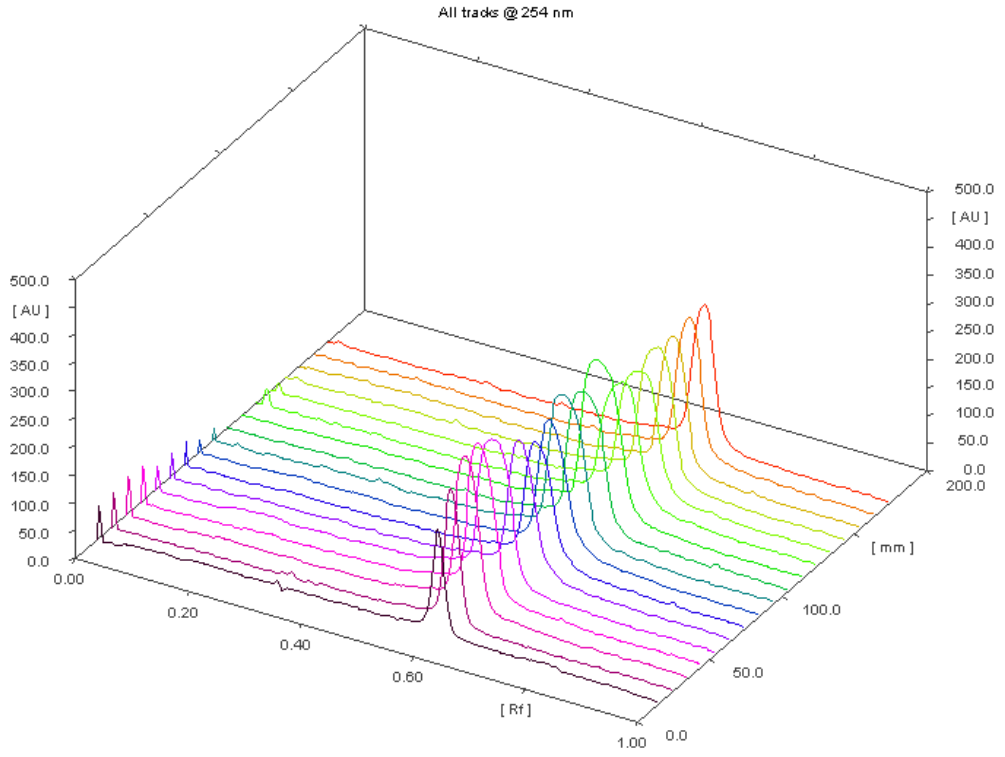


Figure 20. 3D TLC-densitometry chromatogram (plate G – sample No. 12-15)

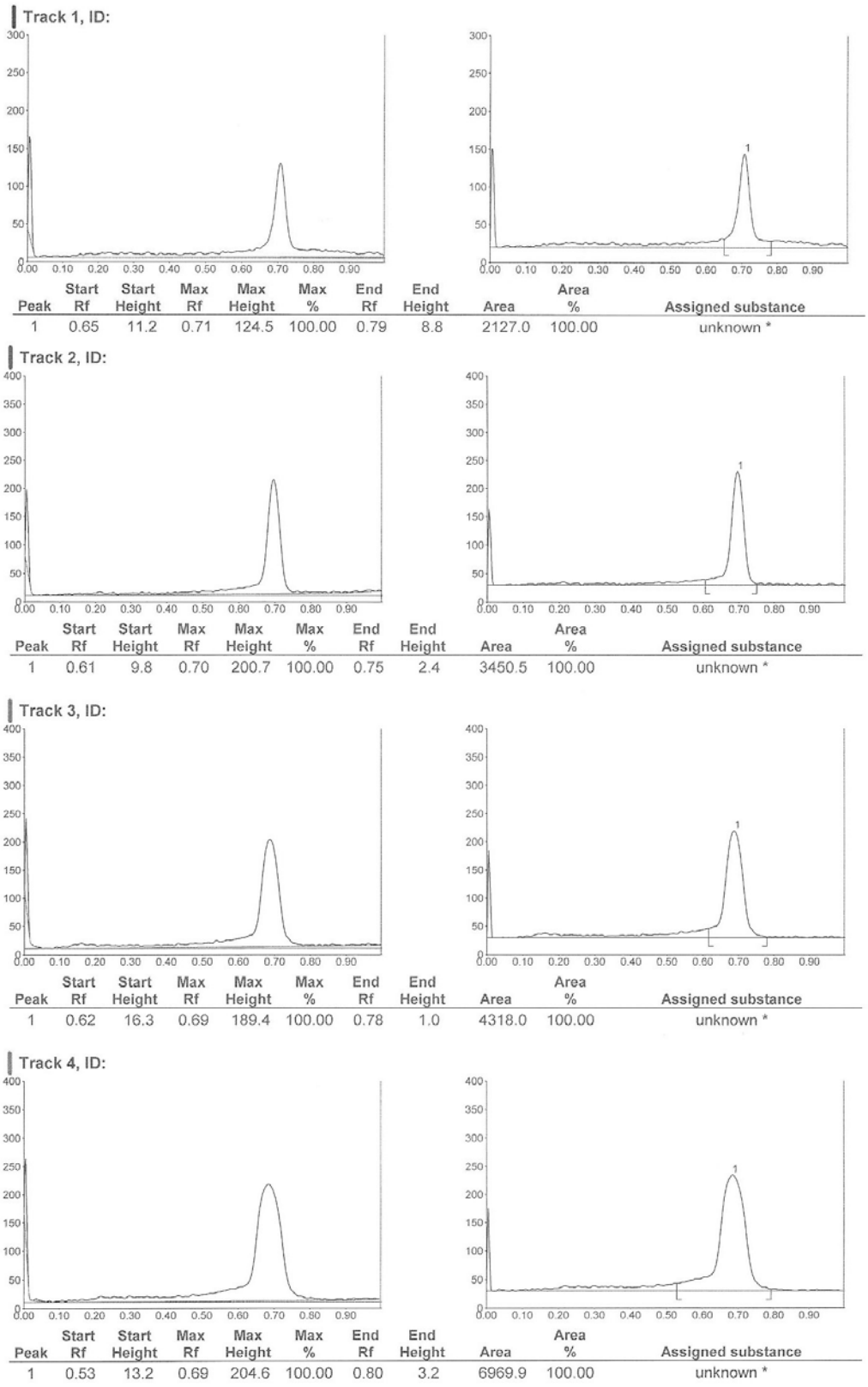
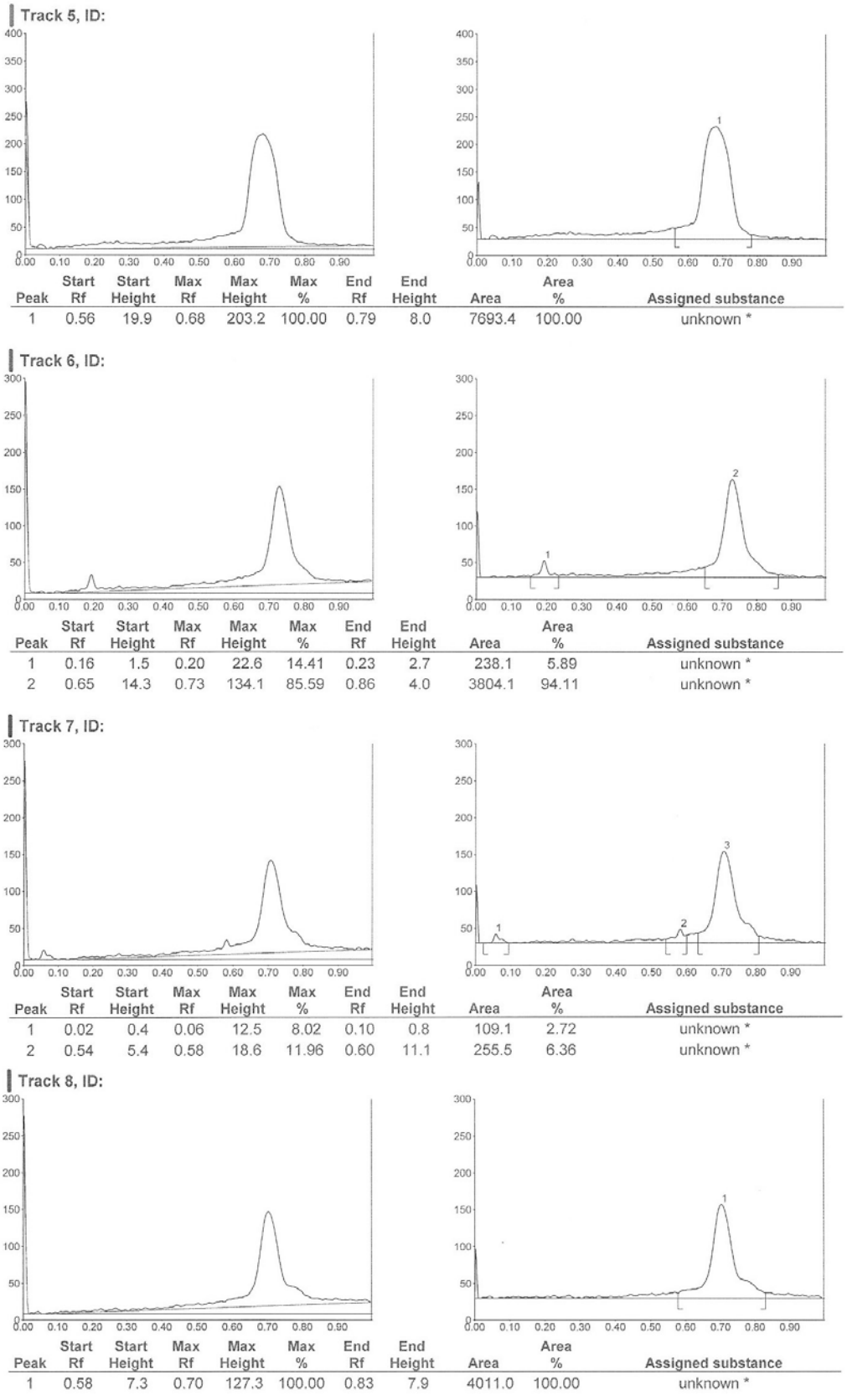


Figure 21. TLC-densitometry chromatogram (plate A–Accuracy)



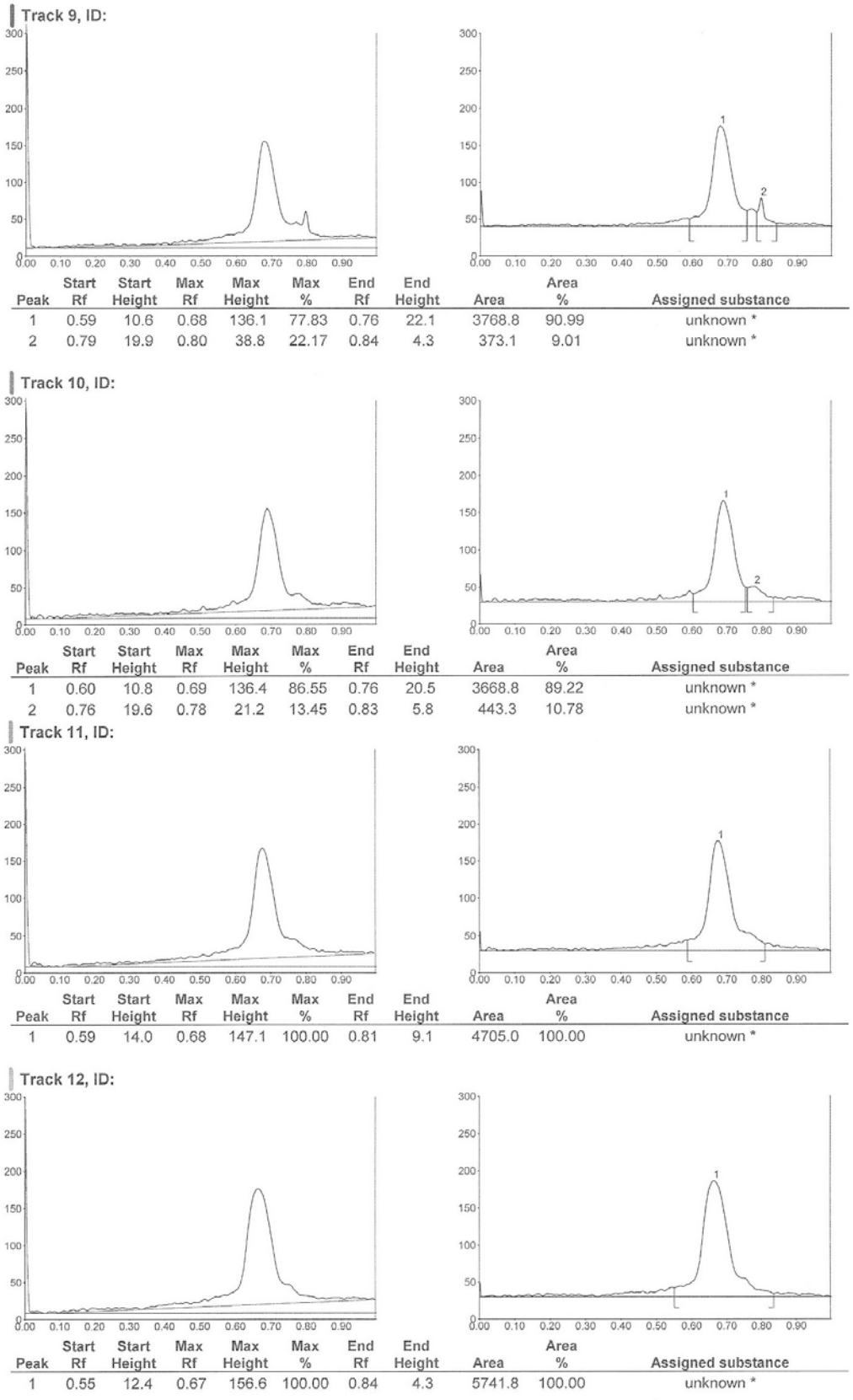
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.56	19.9	0.68	203.2	100.00	0.79	8.0	7693.4	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.16	1.5	0.20	22.6	14.41	0.23	2.7	238.1	5.89	unknown *
2	0.65	14.3	0.73	134.1	85.59	0.86	4.0	3804.1	94.11	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.4	0.06	12.5	8.02	0.10	0.8	109.1	2.72	unknown *
2	0.54	5.4	0.58	18.6	11.96	0.60	11.1	255.5	6.36	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	7.3	0.70	127.3	100.00	0.83	7.9	4011.0	100.00	unknown *

Figure 21 (Cont.). TLC-densitometry chromatogram (plate A–Accuracy)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	10.6	0.68	136.1	77.83	0.76	22.1	3768.8	90.99	unknown *
2	0.79	19.9	0.80	38.8	22.17	0.84	4.3	373.1	9.01	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.60	10.8	0.69	136.4	86.55	0.76	20.5	3668.8	89.22	unknown *
2	0.76	19.6	0.78	21.2	13.45	0.83	5.8	443.3	10.78	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	14.0	0.68	147.1	100.00	0.81	9.1	4705.0	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.55	12.4	0.67	156.6	100.00	0.84	4.3	5741.8	100.00	unknown *

Figure 21 (Cont.). TLC-densitometry chromatogram (plate A–Accuracy)

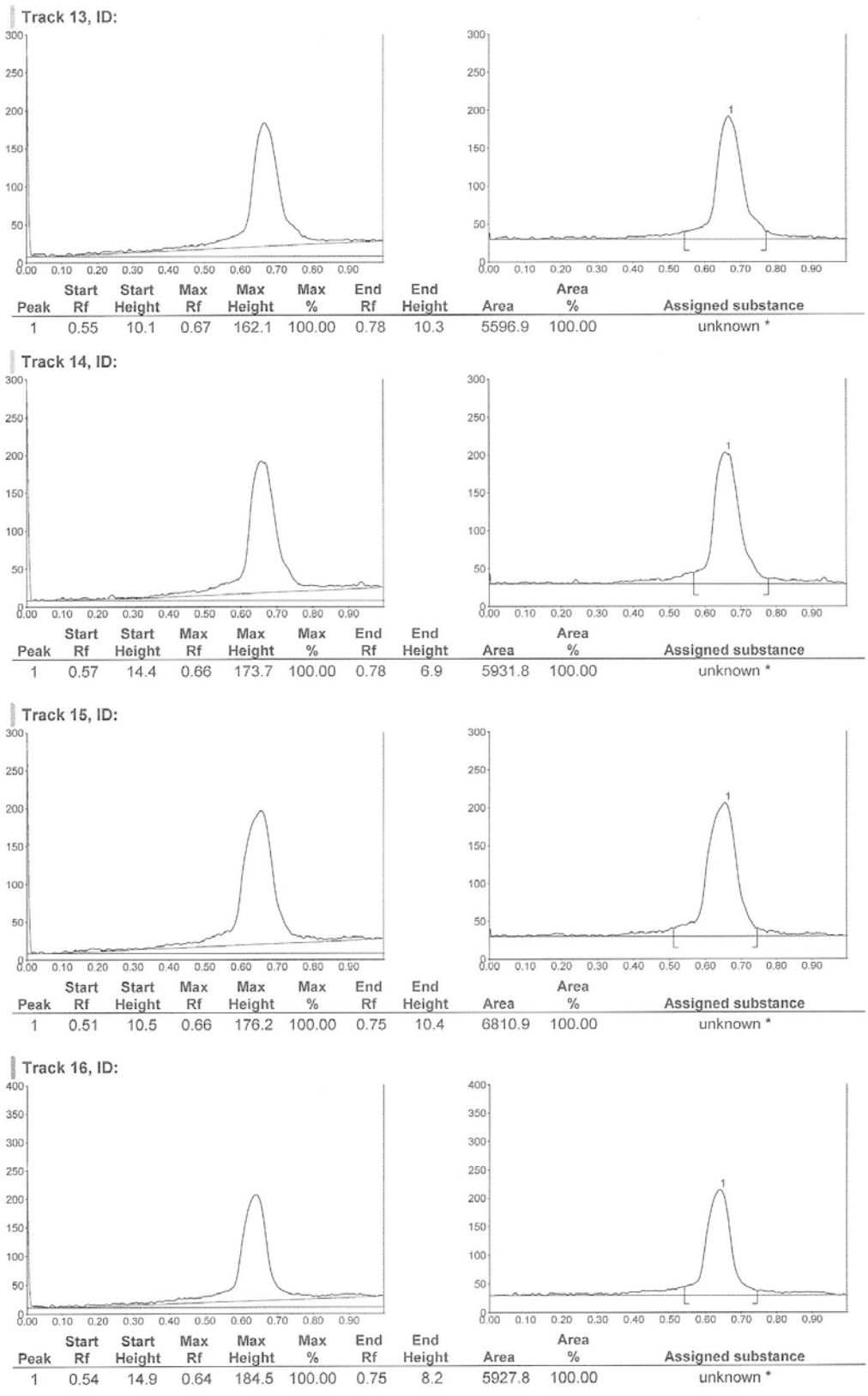
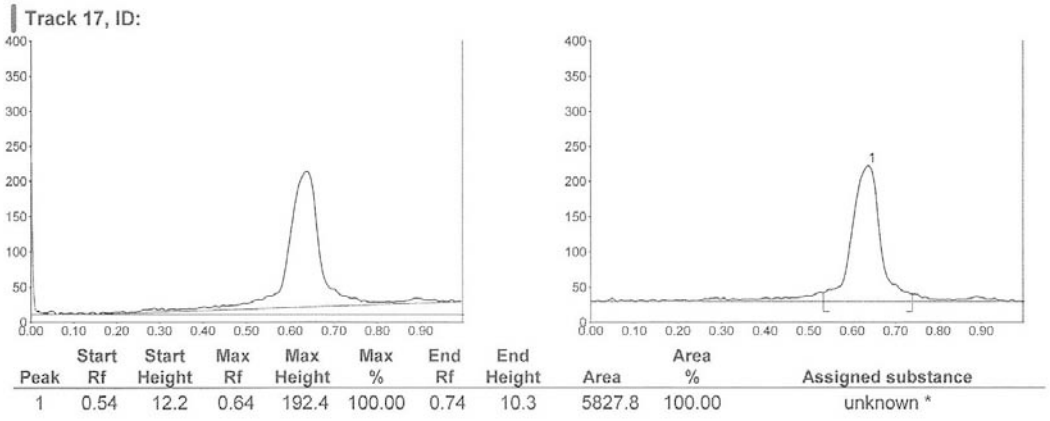


Figure 21 (Cont.). TLC-densitometry chromatogram (plate A–Accuracy)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.54	12.2	0.64	192.4	100.00	0.74	10.3	5827.8	100.00	unknown *

Figure 21 (Cont.). TLC-densitometry chromatogram (plate A–Accuracy)

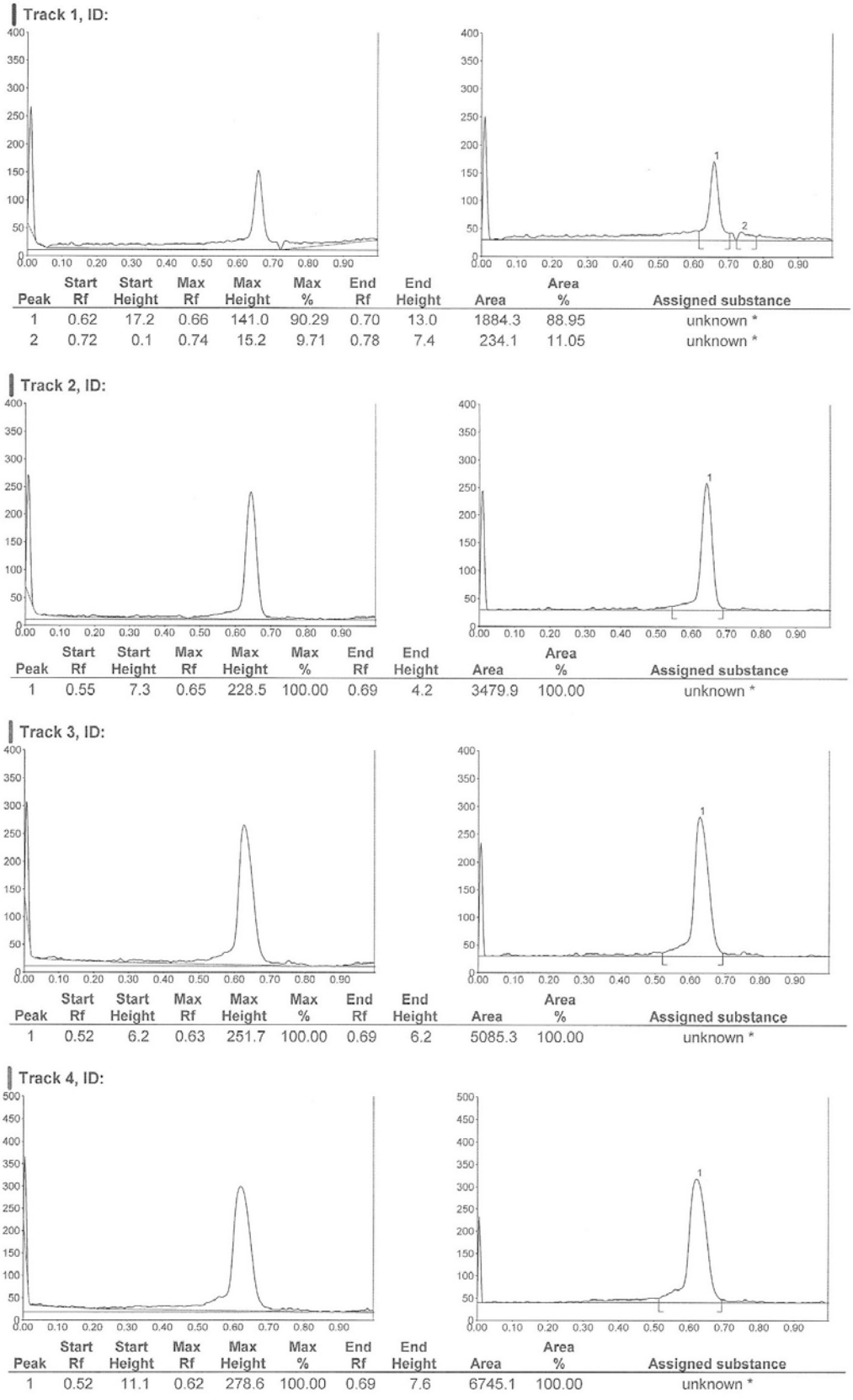


Figure 22. TLC-densitometry chromatogram (plate B –Precision & sample No.1)

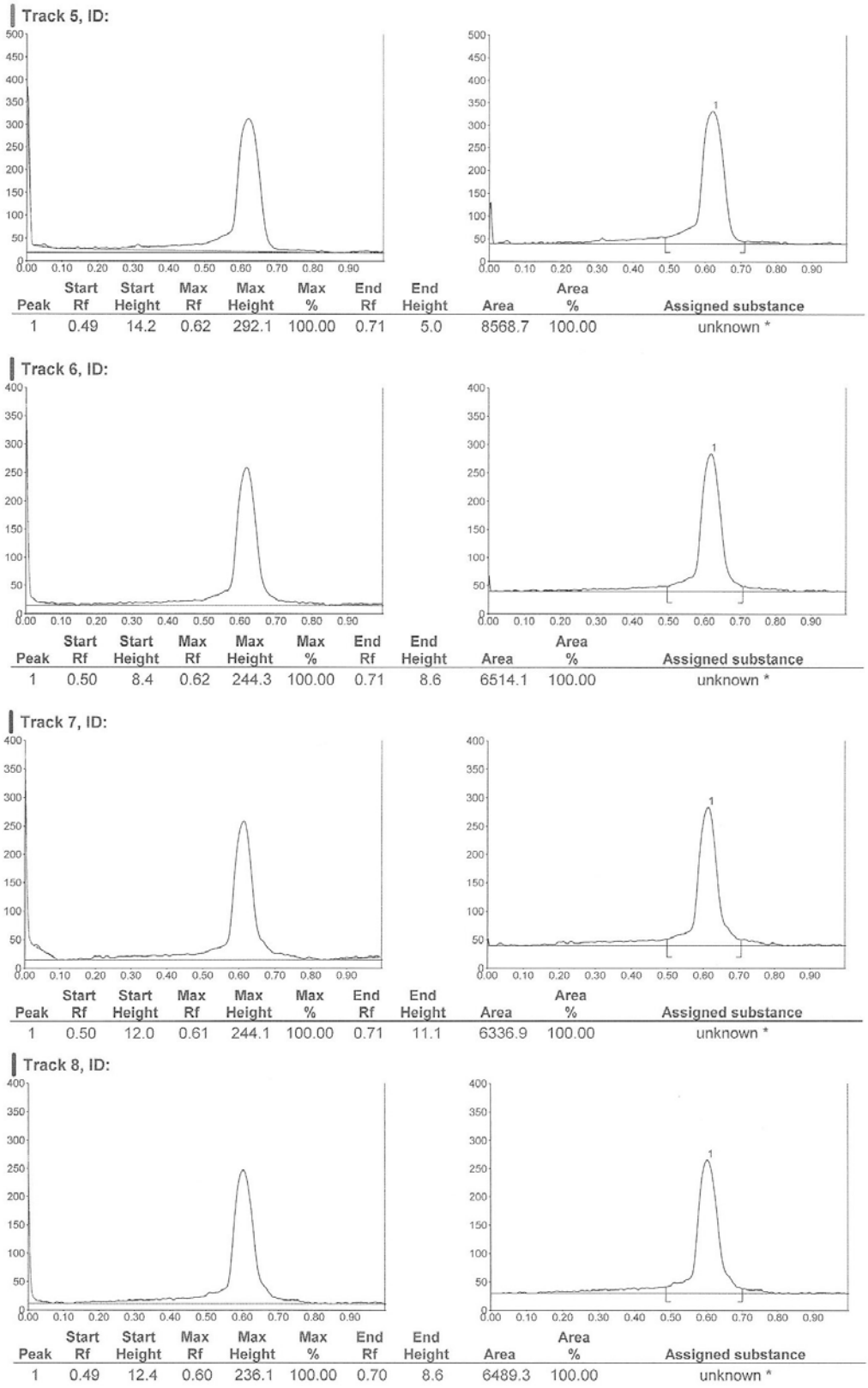


Figure 22 (Cont.). TLC-densitometry chromatogram (plate B –Precision & sample No.1)

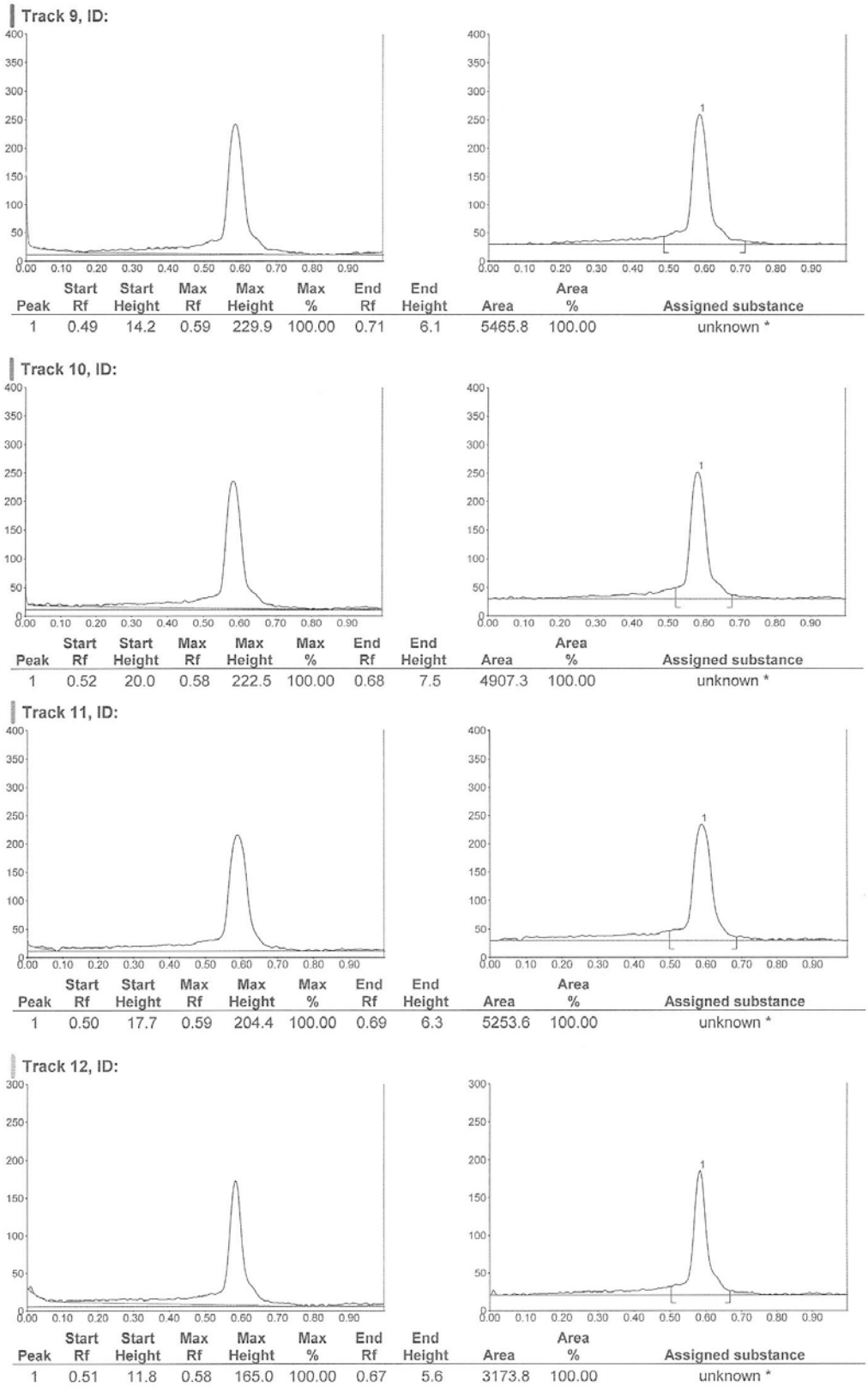
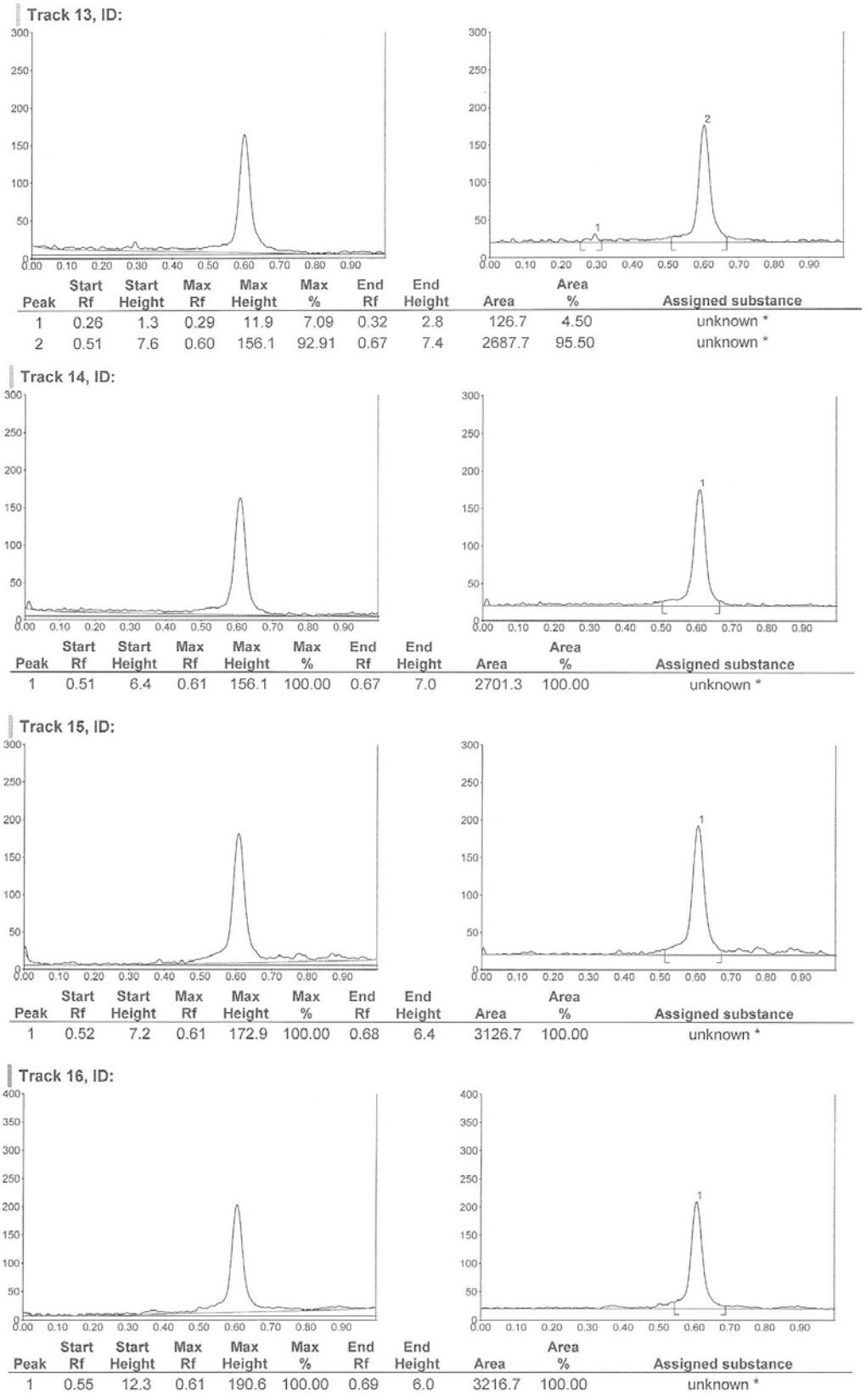


Figure 22 (Cont.). TLC-densitometry chromatogram (plate B –Precision & sample No.1)



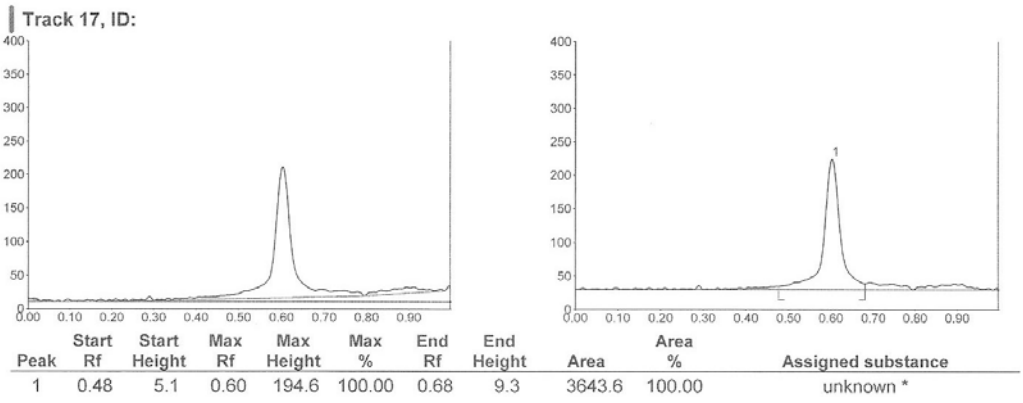
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.26	1.3	0.29	11.9	7.09	0.32	2.8	126.7	4.50	unknown *
2	0.51	7.6	0.60	156.1	92.91	0.67	7.4	2687.7	95.50	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.51	6.4	0.61	156.1	100.00	0.67	7.0	2701.3	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.52	7.2	0.61	172.9	100.00	0.68	6.4	3126.7	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.55	12.3	0.61	190.6	100.00	0.69	6.0	3216.7	100.00	unknown *

Figure 22 (Cont.). TLC-densitometry chromatogram (plate B –Precision & sample No.1)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.48	5.1	0.60	194.6	100.00	0.68	9.3	3643.6	100.00	unknown *

Figure 22 (Cont.). TLC-densitometry chromatogram (plate B –Precision & sample No.1)

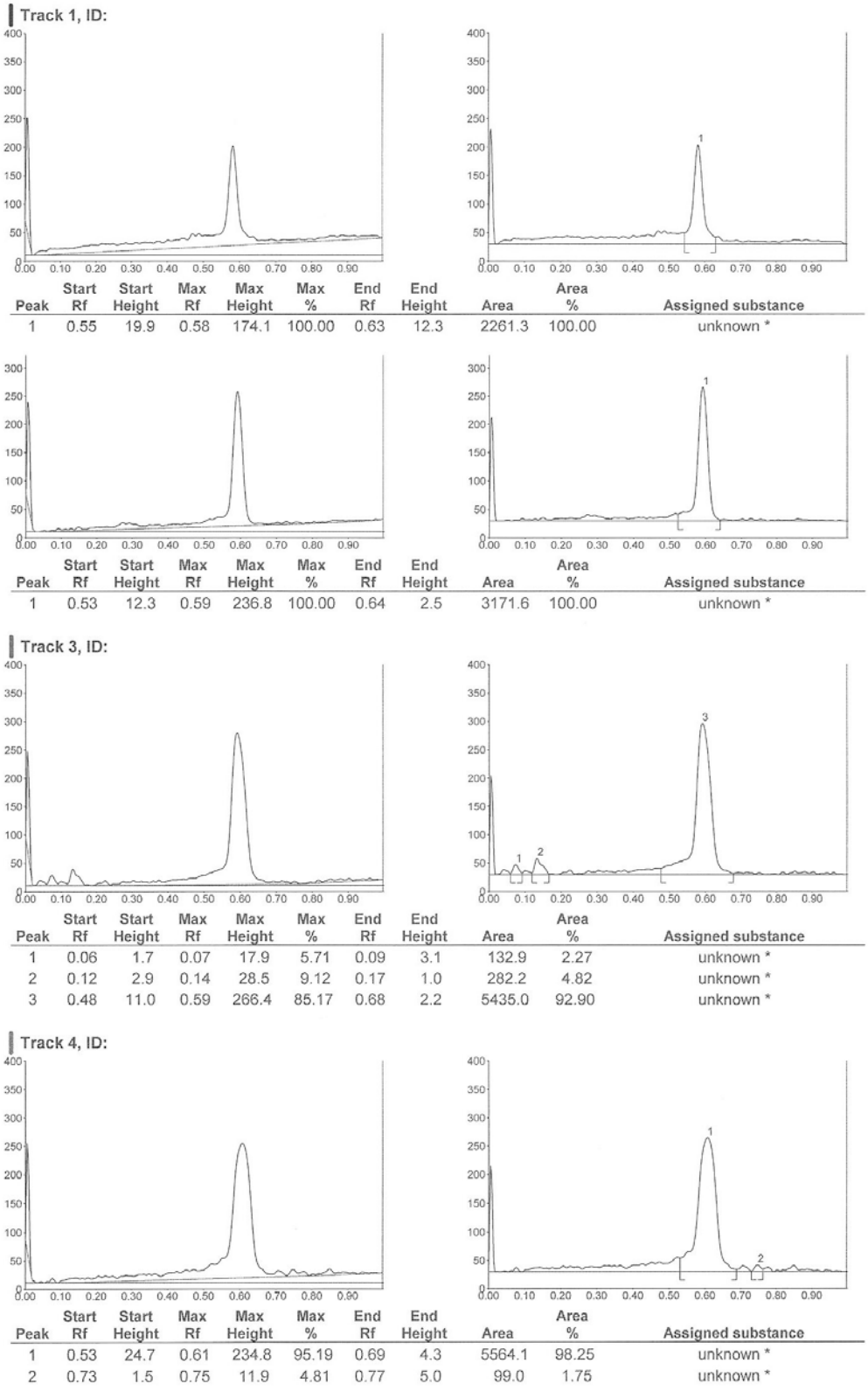
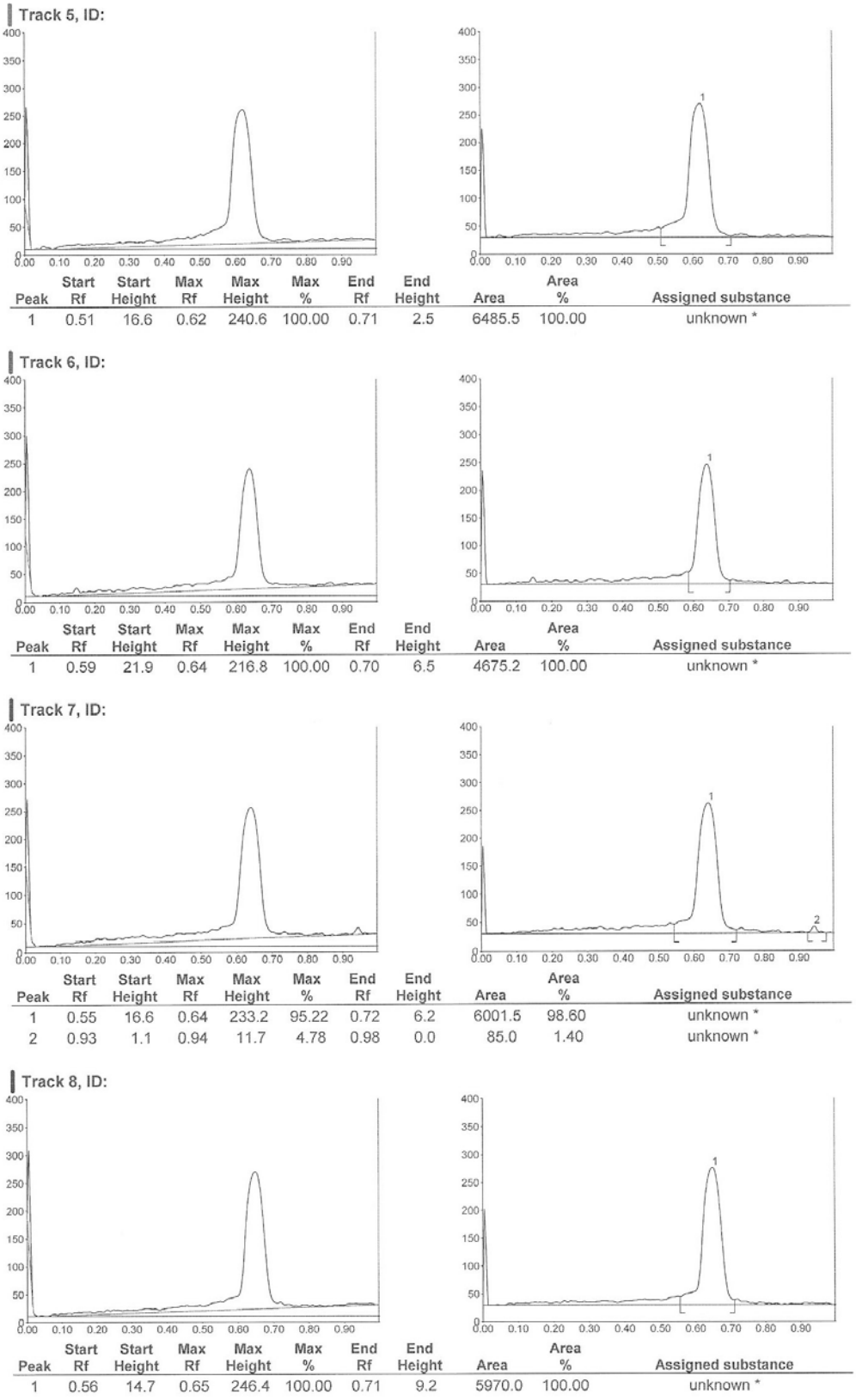
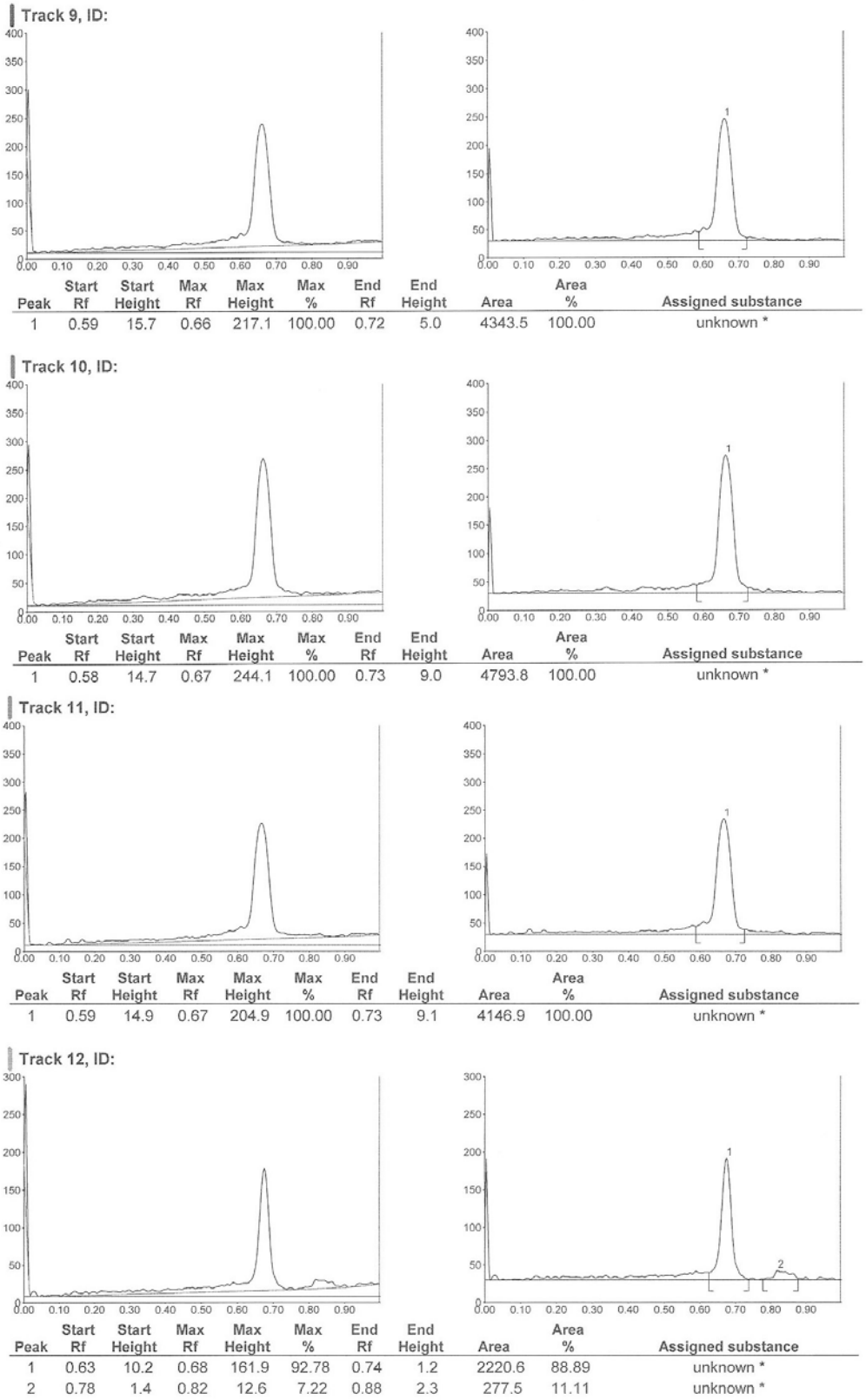


Figure 23. TLC-densitometry chromatogram (plate C – Precision & sample No.2)



Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
5	1	0.51	16.6	0.62	240.6	100.00	0.71	2.5	6485.5	100.00	unknown *
6	1	0.59	21.9	0.64	216.8	100.00	0.70	6.5	4675.2	100.00	unknown *
7	1	0.55	16.6	0.64	233.2	95.22	0.72	6.2	6001.5	98.60	unknown *
7	2	0.93	1.1	0.94	11.7	4.78	0.98	0.0	85.0	1.40	unknown *
8	1	0.56	14.7	0.65	246.4	100.00	0.71	9.2	5970.0	100.00	unknown *

Figure 23 (Cont.). TLC-densitometry chromatogram (plate C – Precision & sample No.2)



Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
9	1	0.59	15.7	0.66	217.1	100.00	0.72	5.0	4343.5	100.00	unknown *
10	1	0.58	14.7	0.67	244.1	100.00	0.73	9.0	4793.8	100.00	unknown *
11	1	0.59	14.9	0.67	204.9	100.00	0.73	9.1	4146.9	100.00	unknown *
12	1	0.63	10.2	0.68	161.9	92.78	0.74	1.2	2220.6	88.89	unknown *
	2	0.78	1.4	0.82	12.6	7.22	0.88	2.3	277.5	11.11	unknown *

Figure 23 (Cont.). TLC-densitometry chromatogram (plate C – Precision & sample No.2)

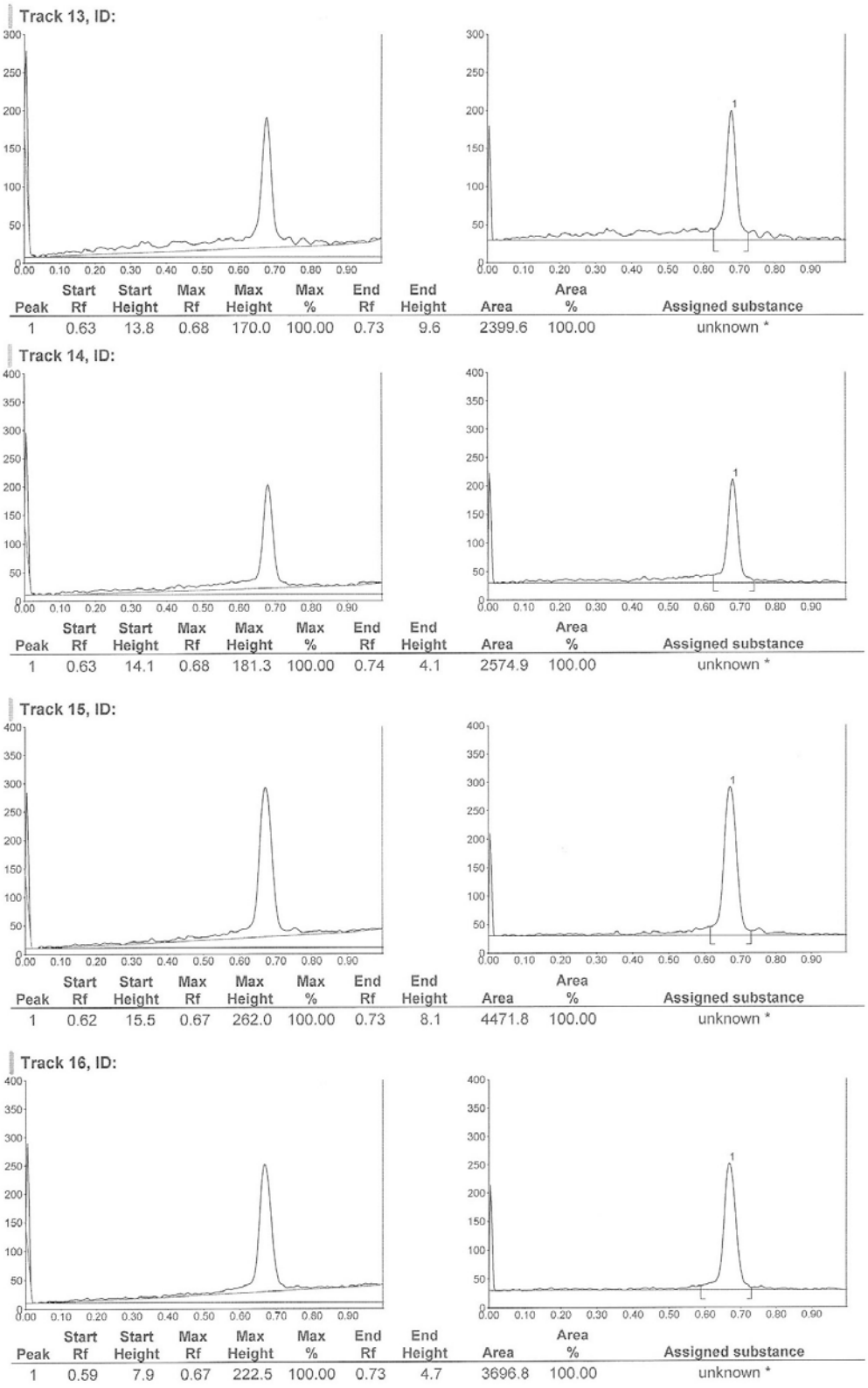
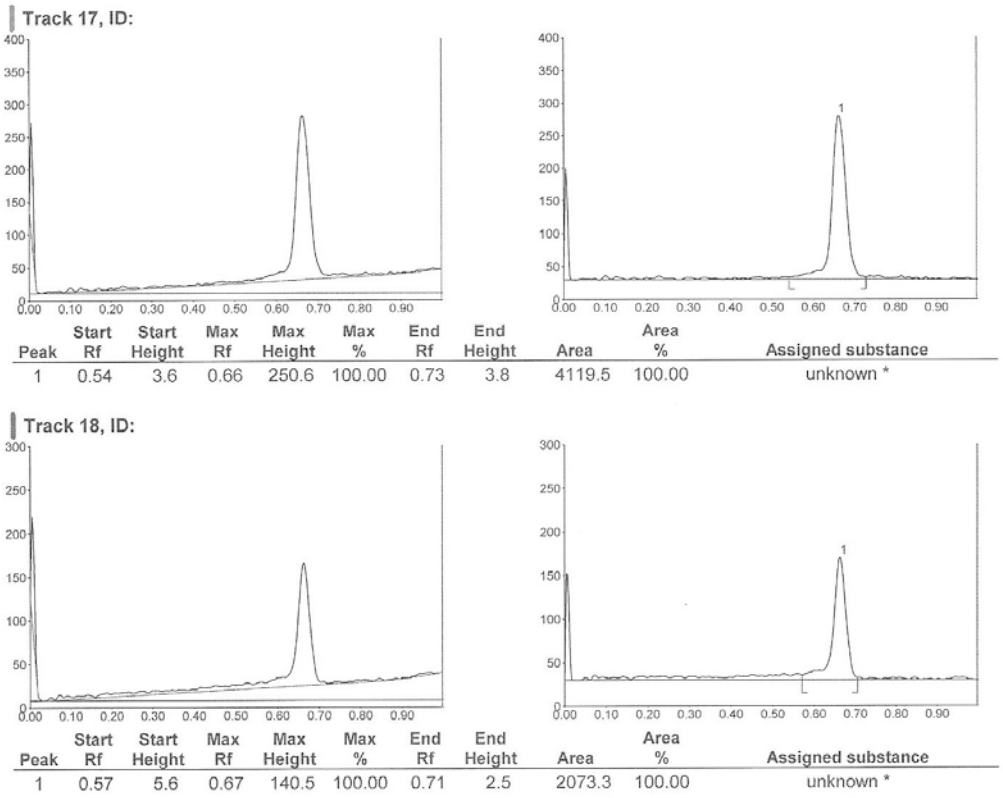


Figure 23 (Cont.). TLC-densitometry chromatogram (plate C – Precision & sample No.2)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.54	3.6	0.66	250.6	100.00	0.73	3.8	4119.5	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	5.6	0.67	140.5	100.00	0.71	2.5	2073.3	100.00	unknown *

Figure 23 (Cont.). TLC-densitometry chromatogram (plate C – Precision & sample No.2)

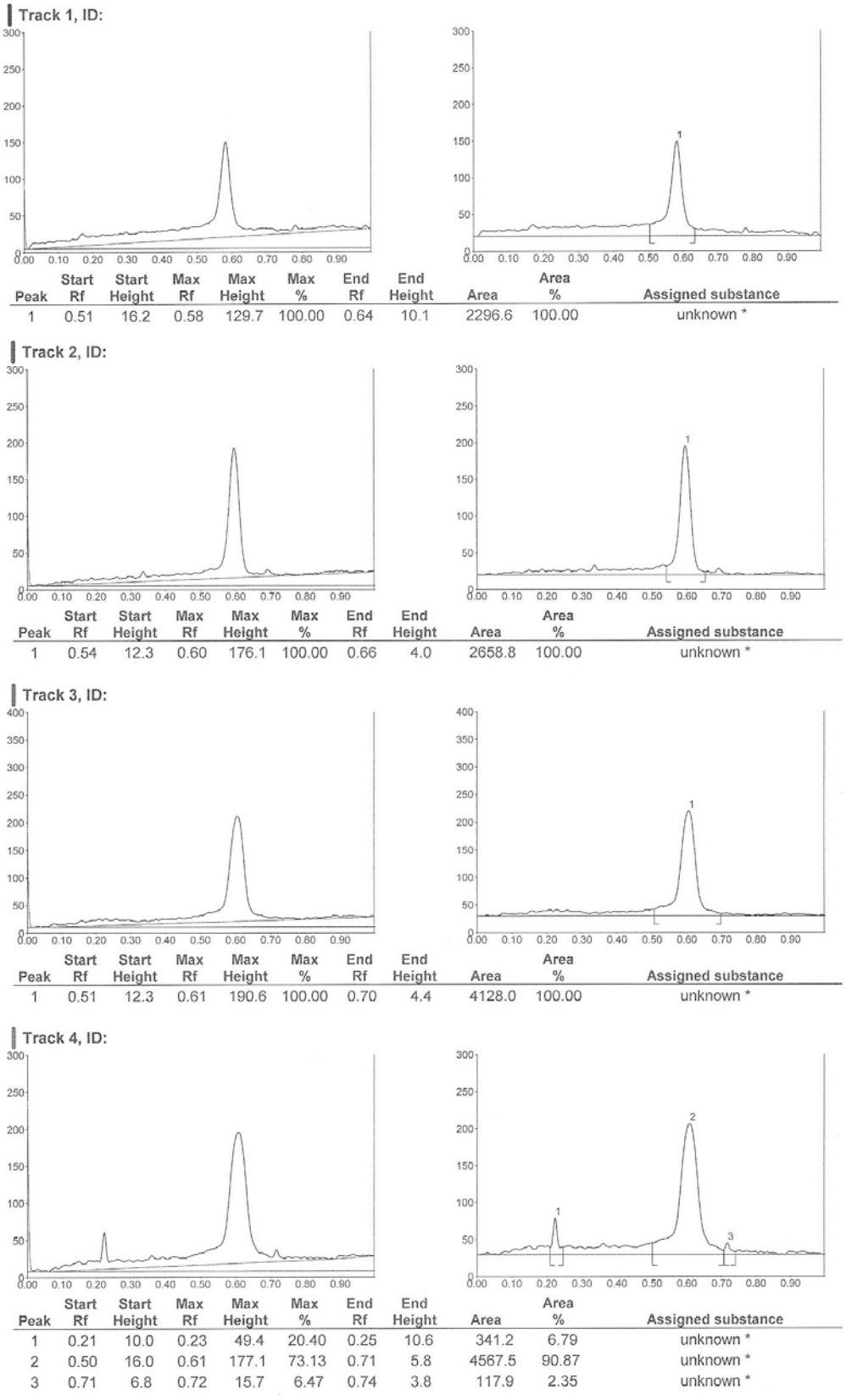


Figure 24. TLC-densitometry chromatogram (plate D – Precision & sample No.3)

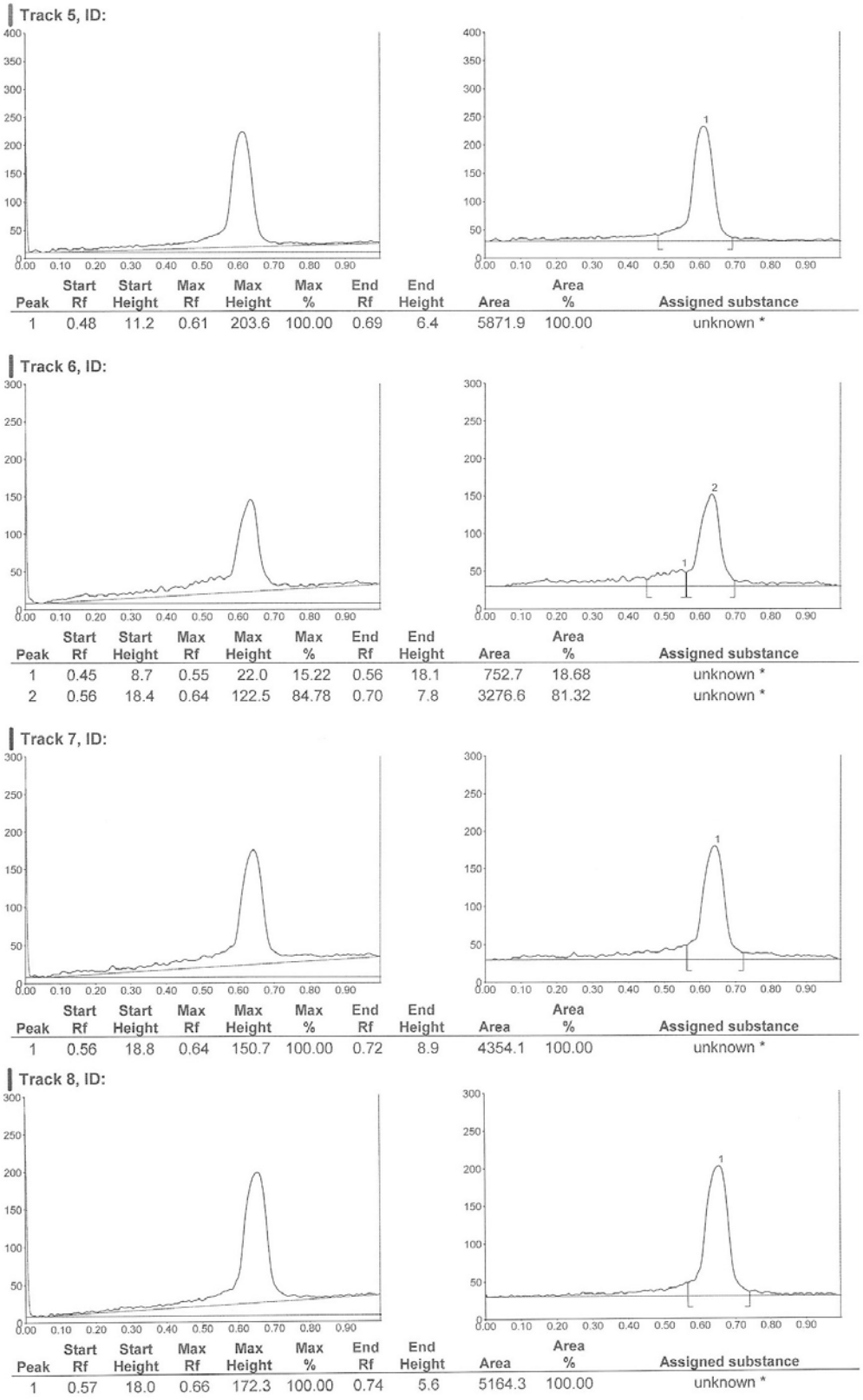


Figure 24 (Cont.). TLC-densitometry chromatogram (plate D – Precision & sample No.3)

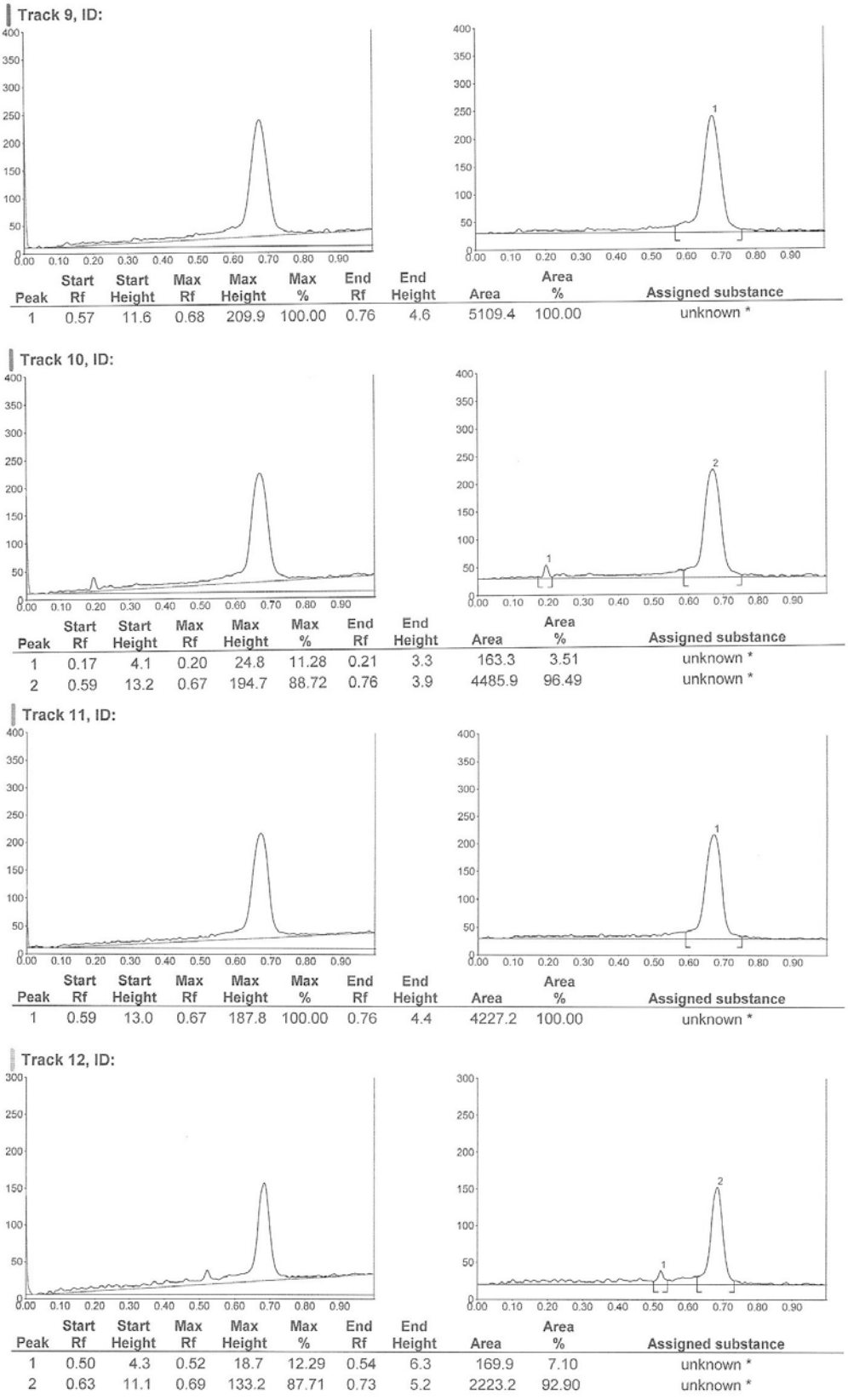


Figure 24 (Cont.). TLC-densitometry chromatogram (plate D – Precision & sample No.3)

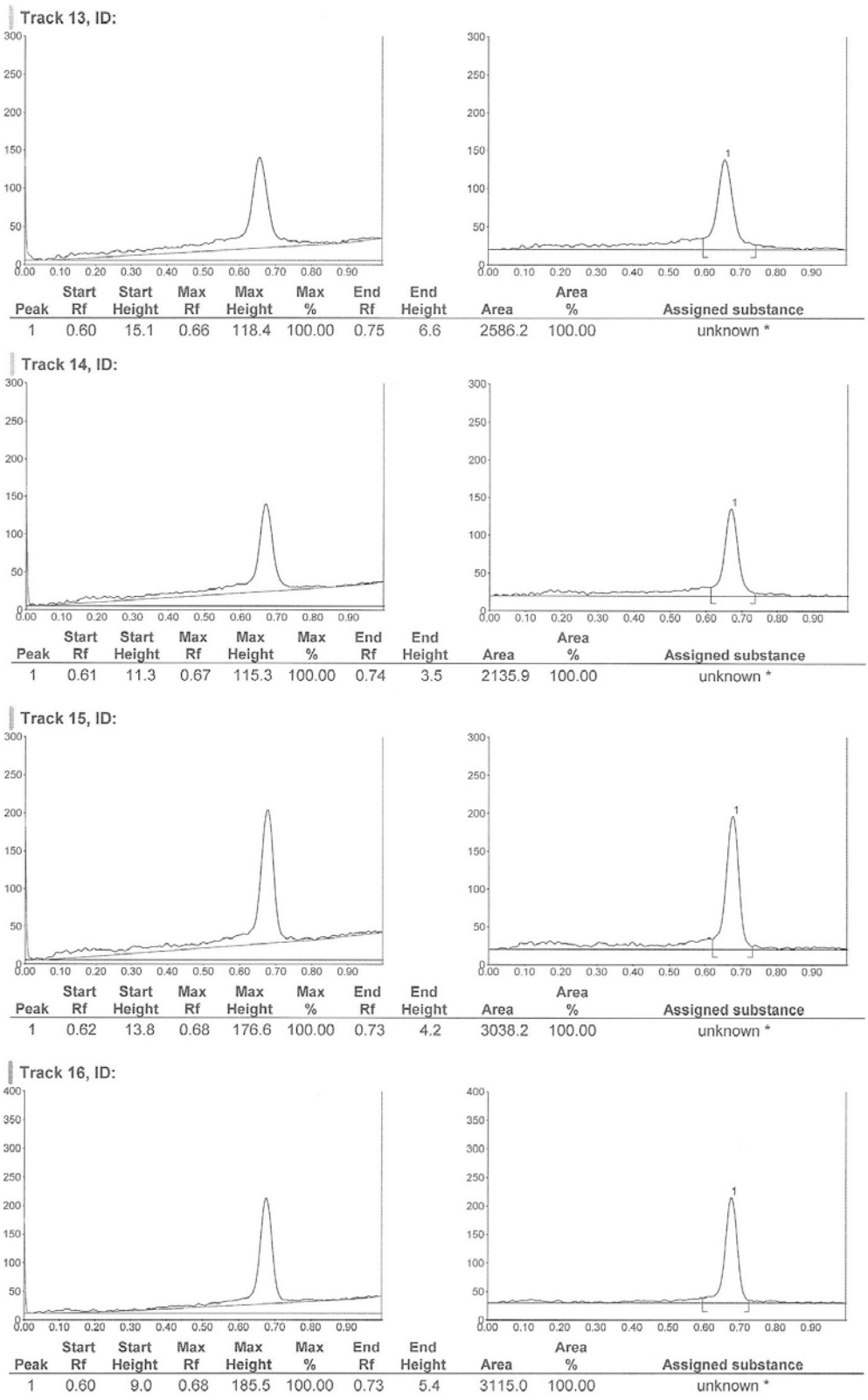
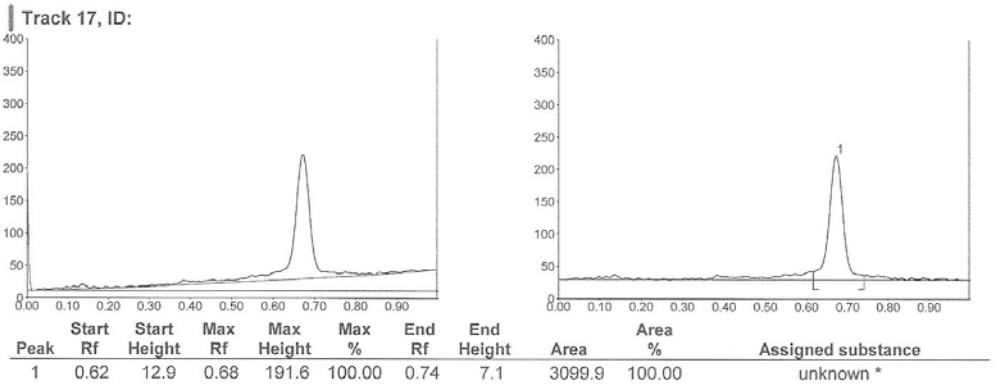
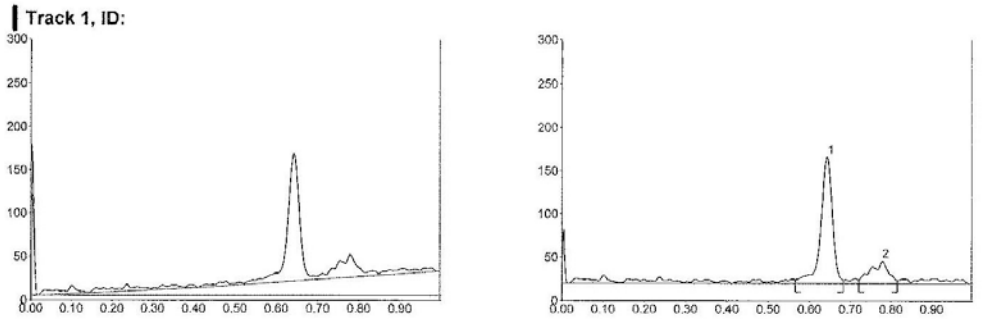


Figure 24 (Cont.). TLC-densitometry chromatogram (plate D – Precision & sample No.3)

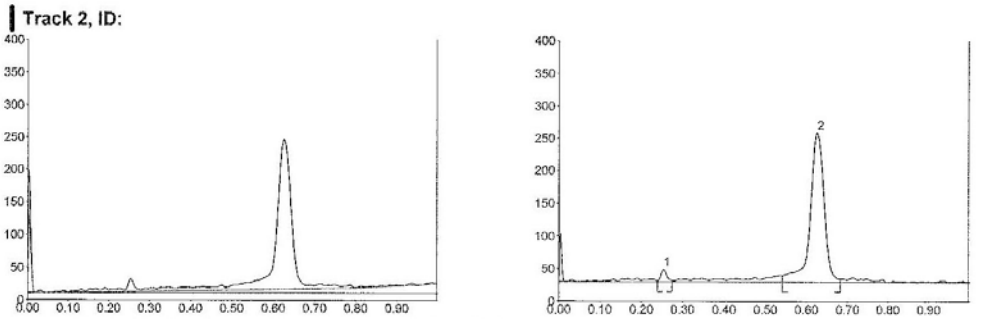


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.62	12.9	0.68	191.6	100.00	0.74	7.1	3099.9	100.00	unknown *

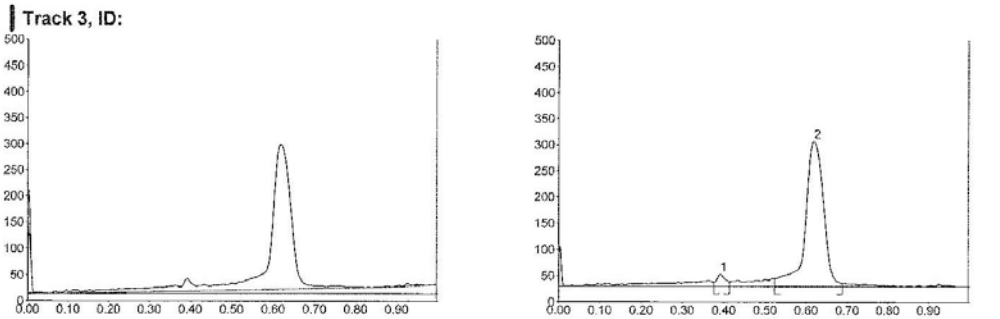
Figure 24 (Cont.). TLC-densitometry chromatogram (plate D – Precision & sample No.3)



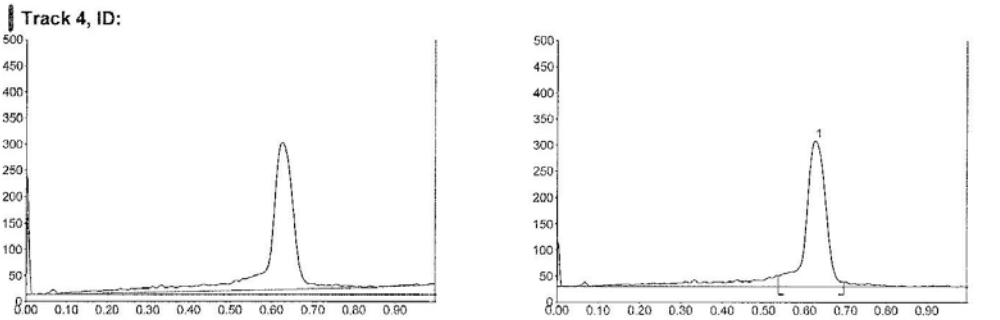
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	4.5	0.65	146.5	85.02	0.69	4.1	1951.2	78.64	unknown *
2	0.72	4.4	0.78	25.8	14.98	0.82	4.0	530.0	21.36	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.24	2.4	0.26	19.5	7.82	0.28	3.1	132.3	3.48	unknown *
2	0.54	10.5	0.63	229.8	92.18	0.69	4.9	3671.8	96.52	unknown *

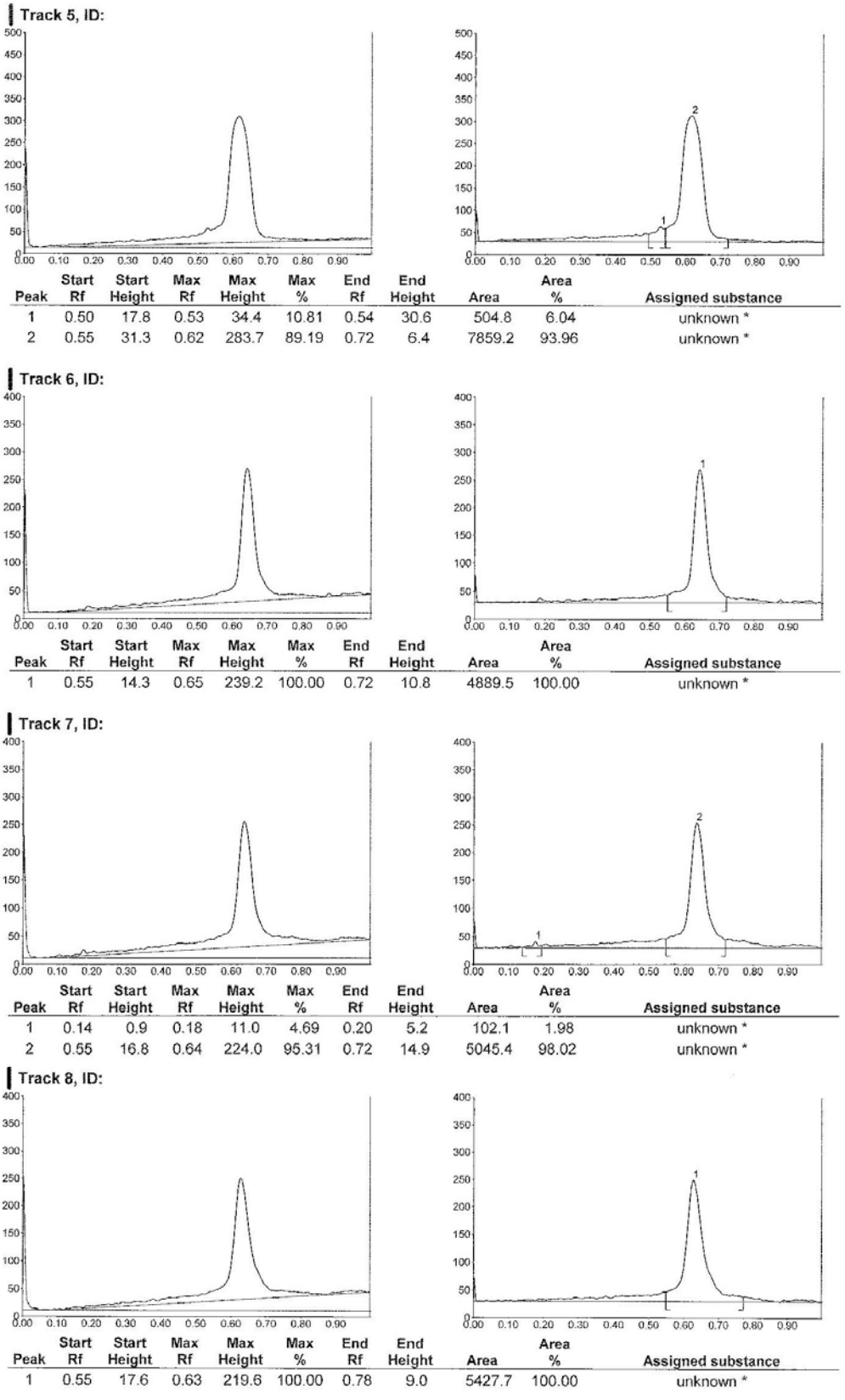


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.38	8.4	0.39	24.1	8.01	0.41	9.1	237.9	3.95	unknown *
2	0.53	15.5	0.62	277.0	91.99	0.69	6.0	5782.1	96.05	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.54	21.6	0.63	279.6	100.00	0.70	9.8	6291.8	100.00	unknown *

Figure 25. TLC-densitometry chromatogram (plate E – sample No. 4-7)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.50	17.8	0.53	34.4	10.81	0.54	30.6	504.8	6.04	unknown *
2	0.55	31.3	0.62	283.7	89.19	0.72	6.4	7859.2	93.96	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.55	14.3	0.65	239.2	100.00	0.72	10.8	4889.5	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.14	0.9	0.18	11.0	4.69	0.20	5.2	102.1	1.98	unknown *
2	0.55	16.8	0.64	224.0	95.31	0.72	14.9	5045.4	98.02	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.55	17.6	0.63	219.6	100.00	0.78	9.0	5427.7	100.00	unknown *

Figure 25 (Cont.). TLC-densitometry chromatogram (plate E – sample No. 4-7)

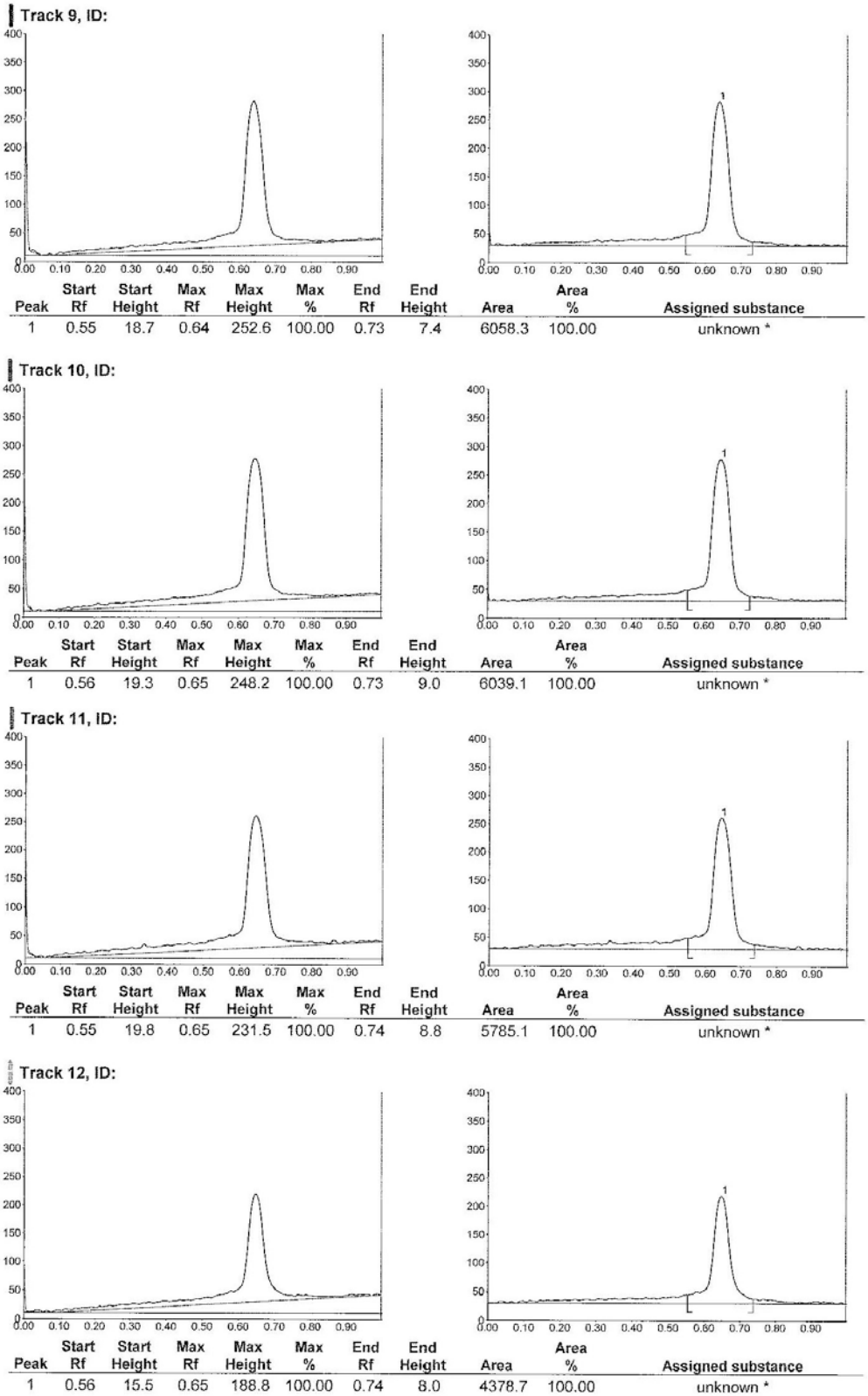
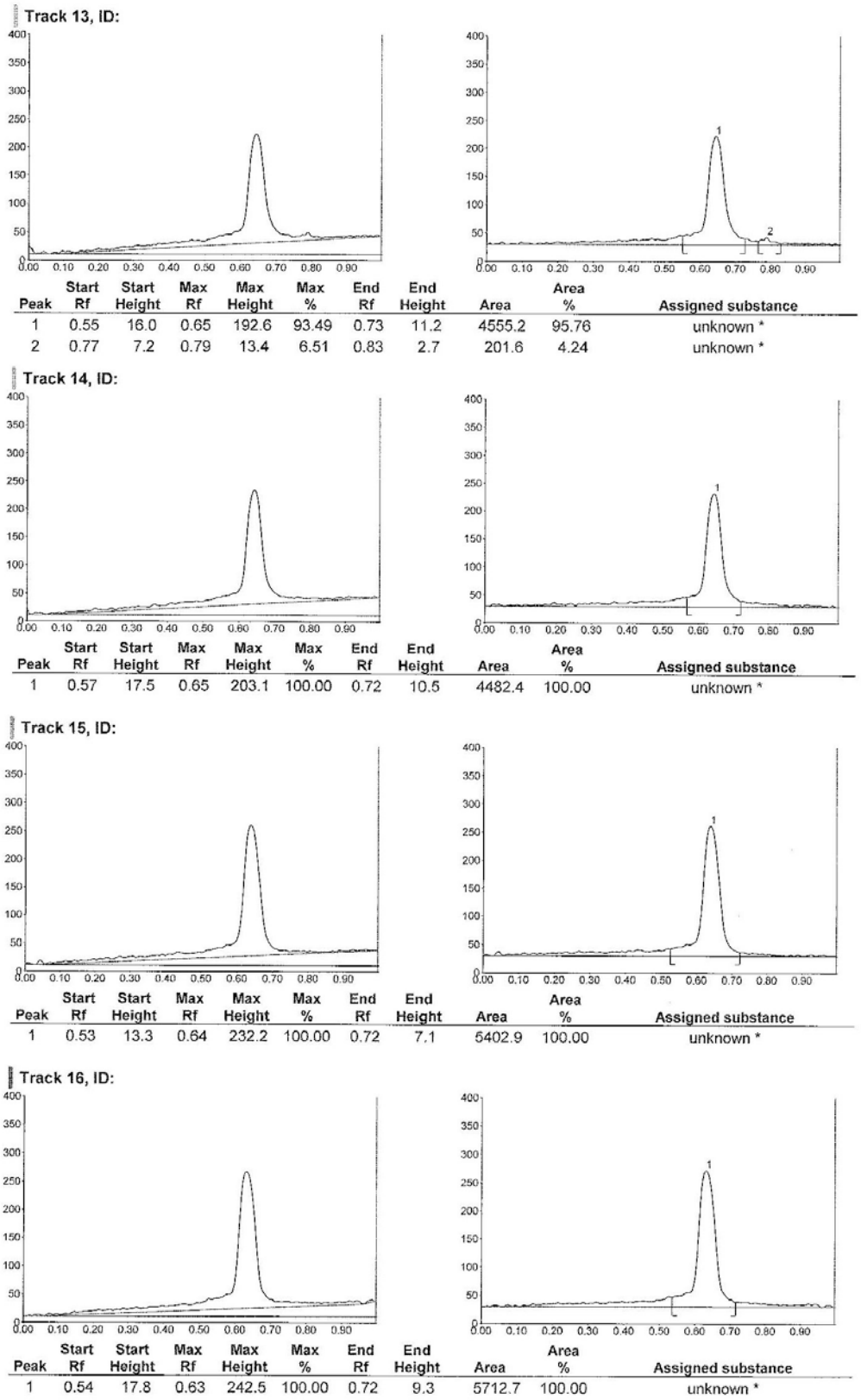
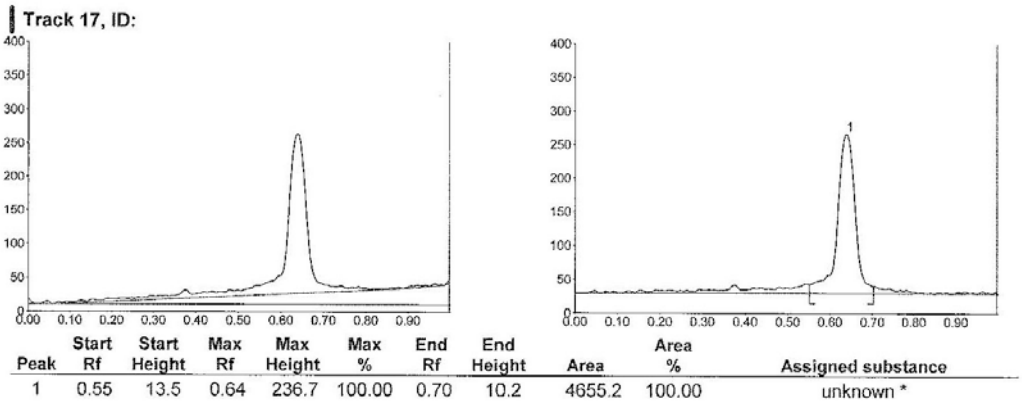


Figure 25 (Cont.). TLC-densitometry chromatogram (plate E – sample No. 4-7)



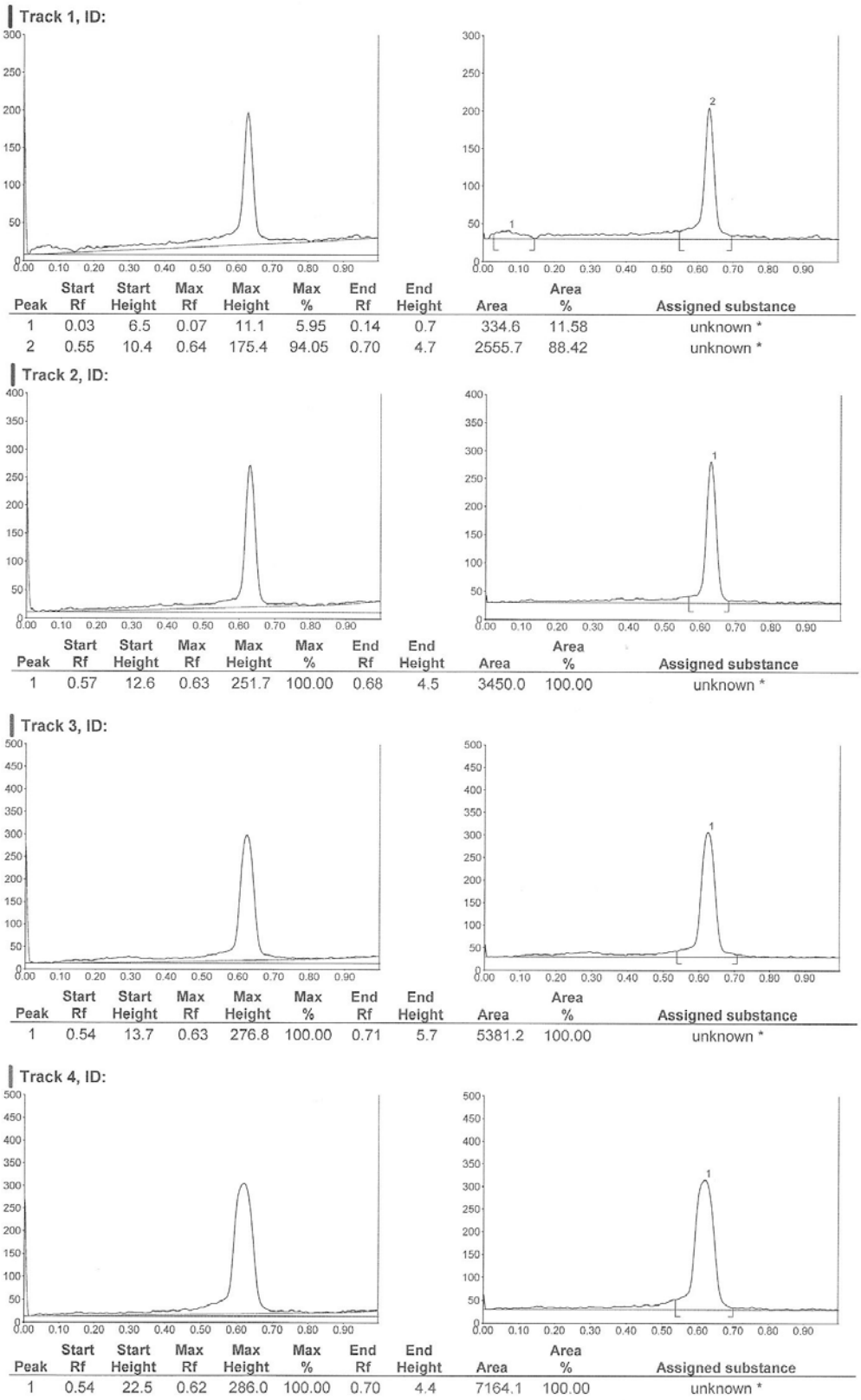
Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
13	1	0.55	16.0	0.65	192.6	93.49	0.73	11.2	4555.2	95.76	unknown *
	2	0.77	7.2	0.79	13.4	6.51	0.83	2.7	201.6	4.24	unknown *
14	1	0.57	17.5	0.65	203.1	100.00	0.72	10.5	4482.4	100.00	unknown *
15	1	0.53	13.3	0.64	232.2	100.00	0.72	7.1	5402.9	100.00	unknown *
16	1	0.54	17.8	0.63	242.5	100.00	0.72	9.3	5712.7	100.00	unknown *

Figure 25 (Cont.). TLC-densitometry chromatogram (plate E – sample No. 4-7)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.55	13.5	0.64	236.7	100.00	0.70	10.2	4655.2	100.00	unknown *

Figure 25 (Cont.). TLC-densitometry chromatogram (plate E – sample No. 4-7)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	6.5	0.07	11.1	5.95	0.14	0.7	334.6	11.58	unknown *
2	0.55	10.4	0.64	175.4	94.05	0.70	4.7	2555.7	88.42	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	12.6	0.63	251.7	100.00	0.68	4.5	3450.0	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.54	13.7	0.63	276.8	100.00	0.71	5.7	5381.2	100.00	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.54	22.5	0.62	286.0	100.00	0.70	4.4	7164.1	100.00	unknown *

Figure 26. TLC-densitometry chromatogram (plate F – sample No. 8-11)

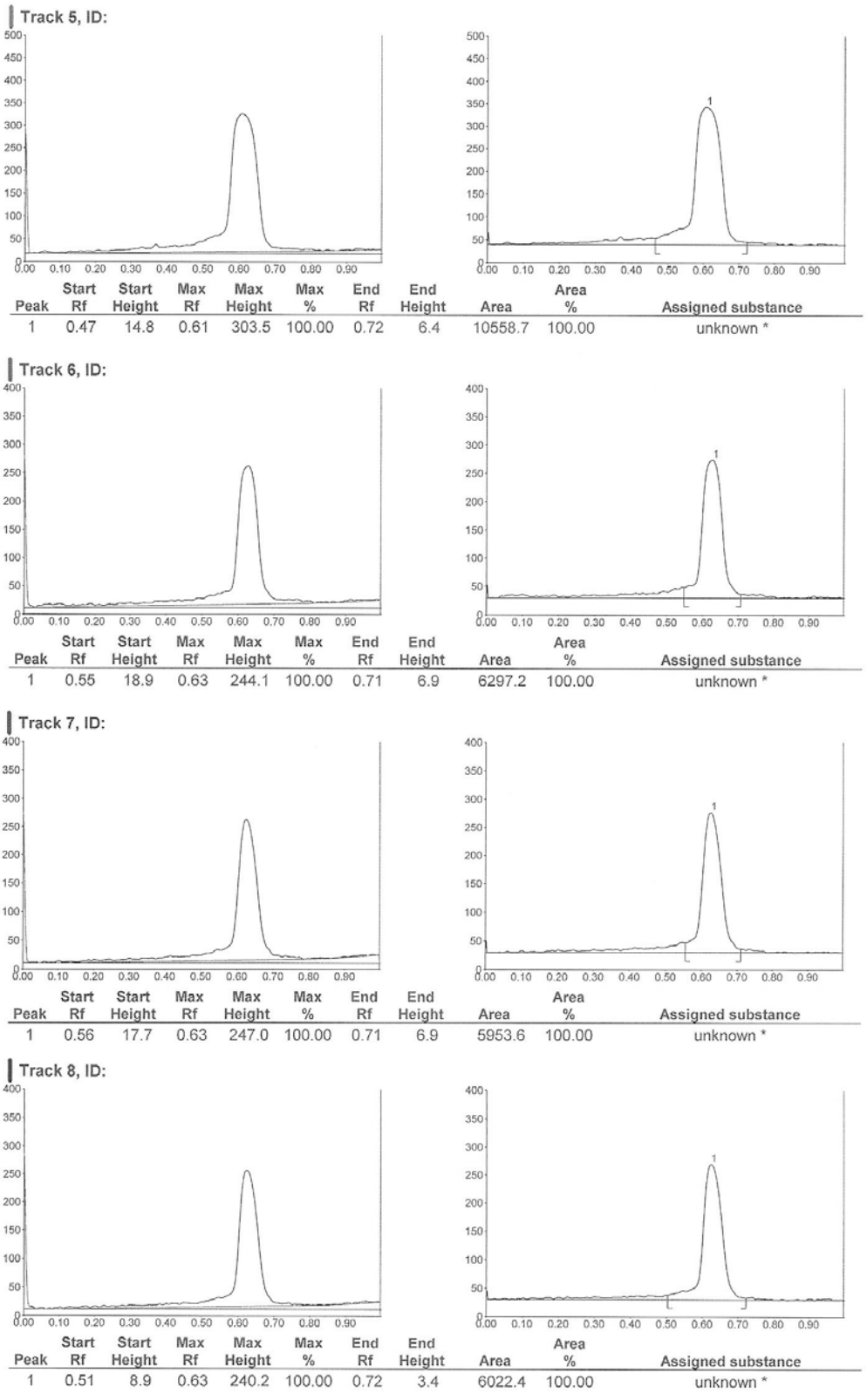


Figure 26 (Cont.). TLC-densitometry chromatogram (plate F – sample No. 8-11)

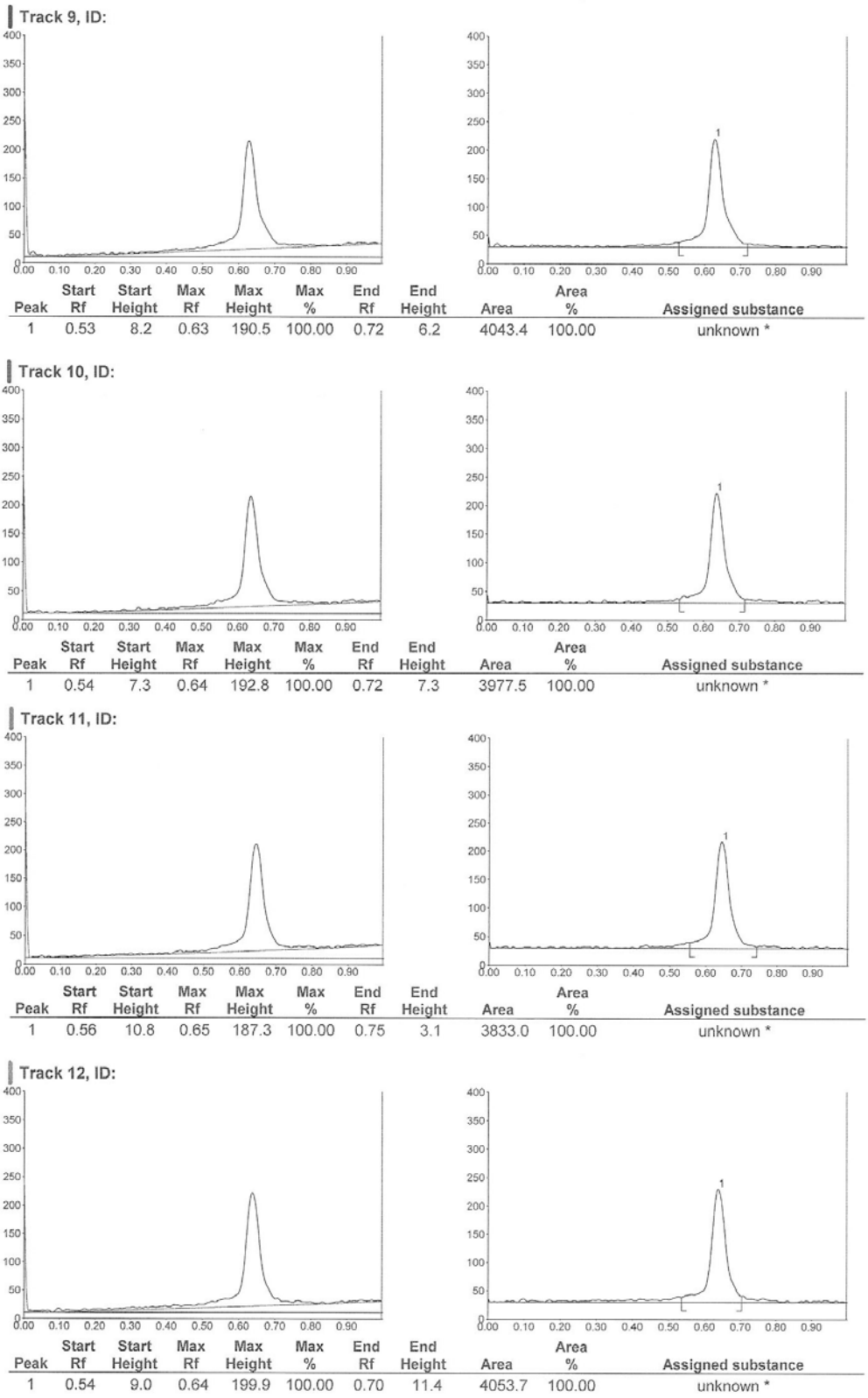


Figure 26 (Cont.). TLC-densitometry chromatogram (plate F – sample No. 8-11)

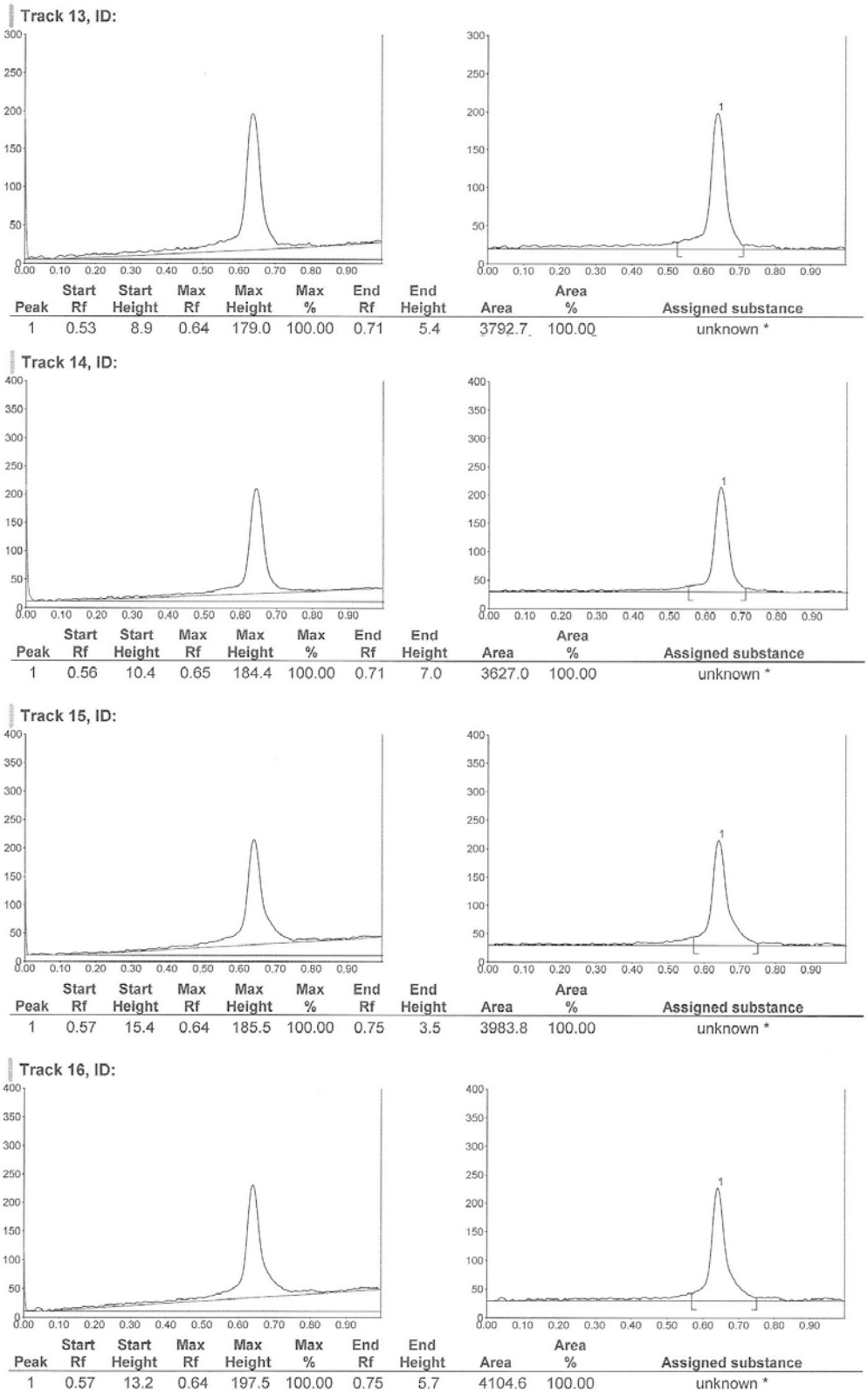
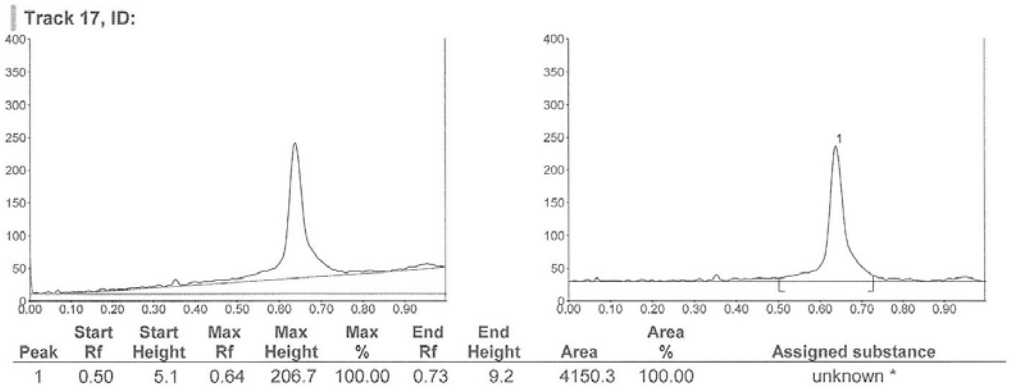
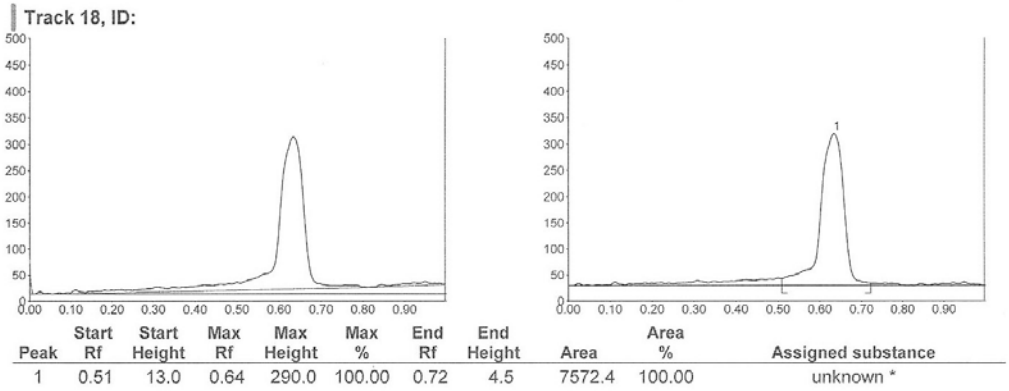


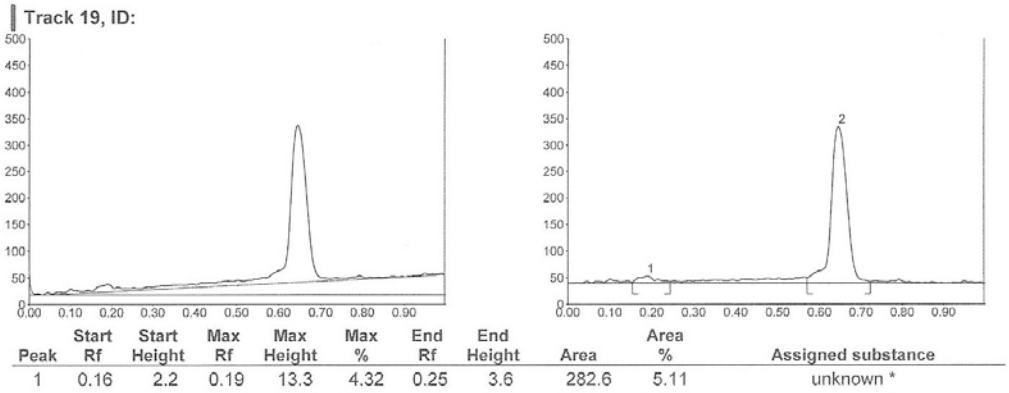
Figure 26 (Cont.). TLC-densitometry chromatogram (plate F – sample No. 8-11)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.50	5.1	0.64	206.7	100.00	0.73	9.2	4150.3	100.00	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.51	13.0	0.64	290.0	100.00	0.72	4.5	7572.4	100.00	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.16	2.2	0.19	13.3	4.32	0.25	3.6	282.6	5.11	unknown *
2	0.57	10.7	0.65	295.6	95.68	0.72	3.4	5252.3	94.89	unknown *

Figure 26 (Cont.). TLC-densitometry chromatogram (plate F – sample No. 8-11)

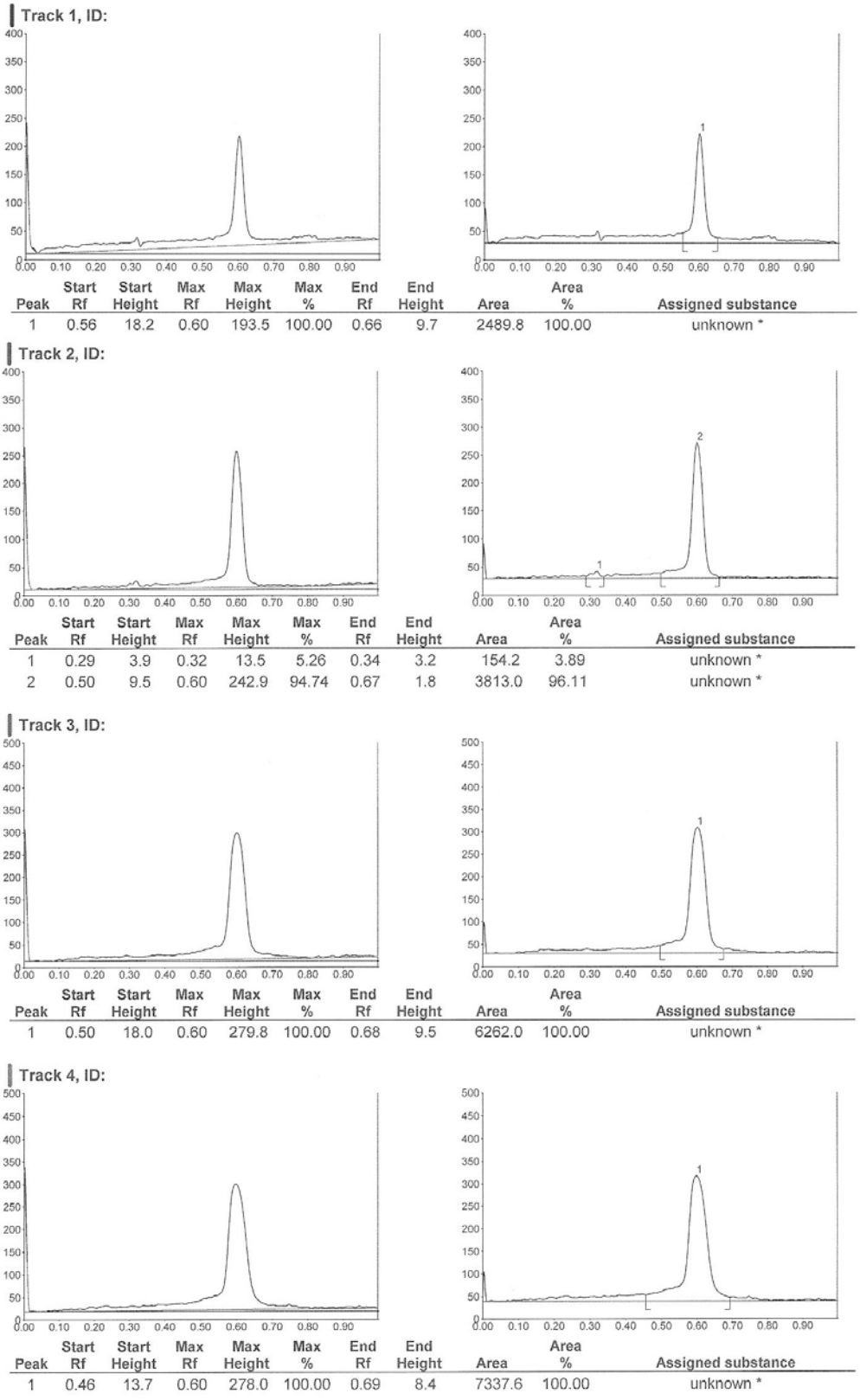
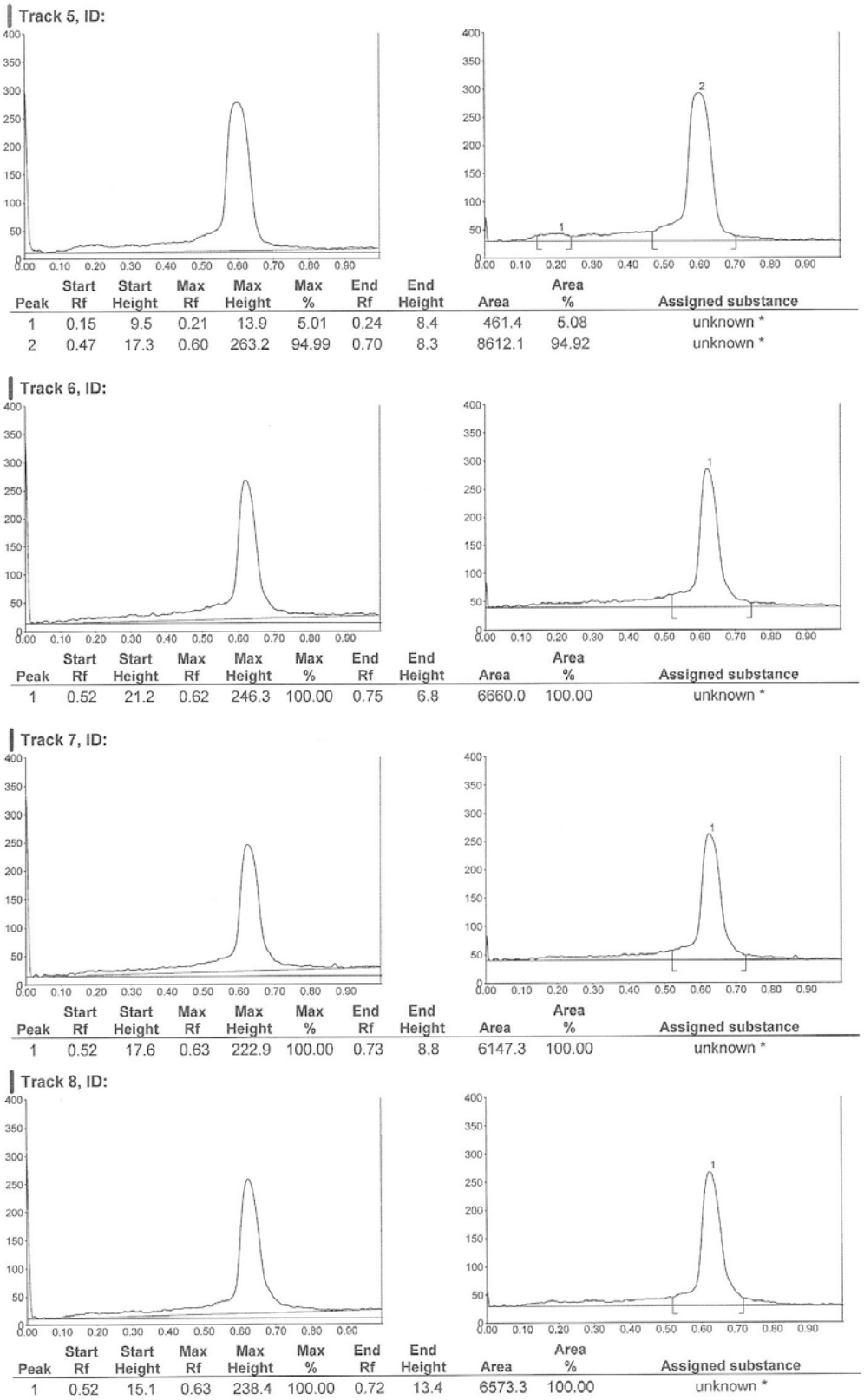
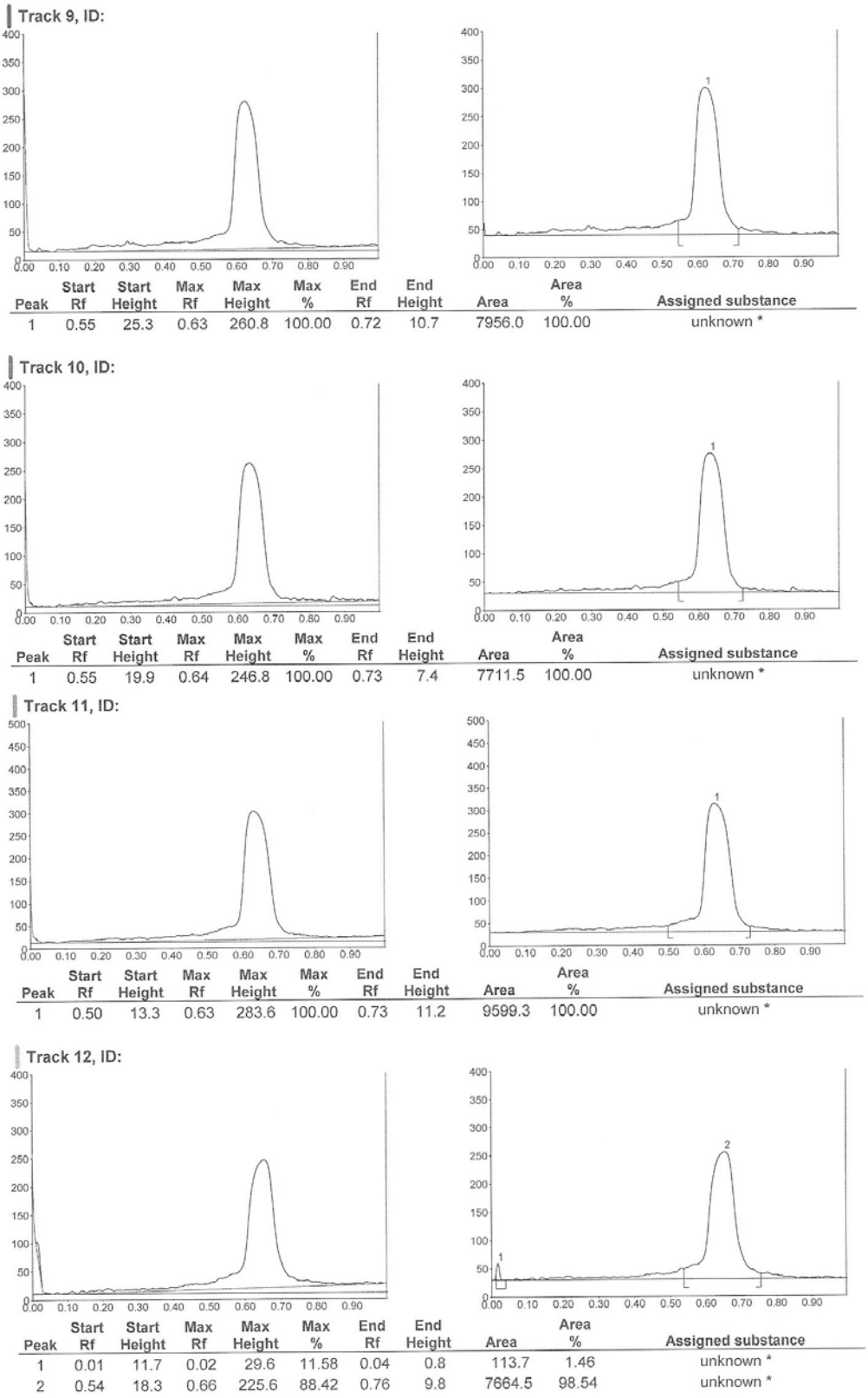


Figure 27. TLC-densitometry chromatogram (plate G – sample No. 12-15)



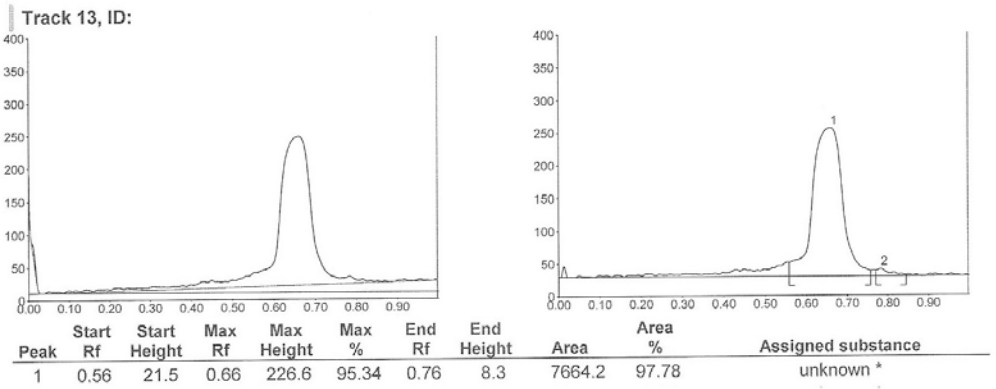
Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
5	1	0.15	9.5	0.21	13.9	5.01	0.24	8.4	461.4	5.08	unknown *
	2	0.47	17.3	0.60	263.2	94.99	0.70	8.3	8612.1	94.92	unknown *
6	1	0.52	21.2	0.62	246.3	100.00	0.75	6.8	6660.0	100.00	unknown *
7	1	0.52	17.6	0.63	222.9	100.00	0.73	8.8	6147.3	100.00	unknown *
8	1	0.52	15.1	0.63	238.4	100.00	0.72	13.4	6573.3	100.00	unknown *

Figure 27 (Cont.). TLC-densitometry chromatogram (plate G – sample No. 12-15)

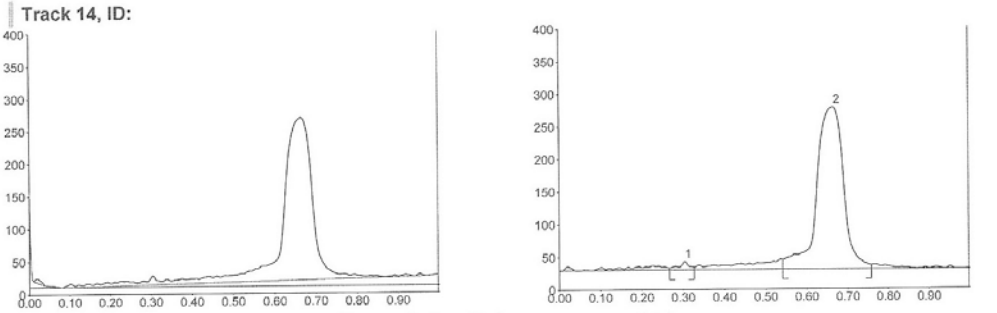


Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
9	1	0.55	25.3	0.63	260.8	100.00	0.72	10.7	7956.0	100.00	unknown *
10	1	0.55	19.9	0.64	246.8	100.00	0.73	7.4	7711.5	100.00	unknown *
11	1	0.50	13.3	0.63	283.6	100.00	0.73	11.2	9599.3	100.00	unknown *
12	1	0.01	11.7	0.02	29.6	11.58	0.04	0.8	113.7	1.46	unknown *
12	2	0.54	18.3	0.66	225.6	88.42	0.76	9.8	7664.5	98.54	unknown *

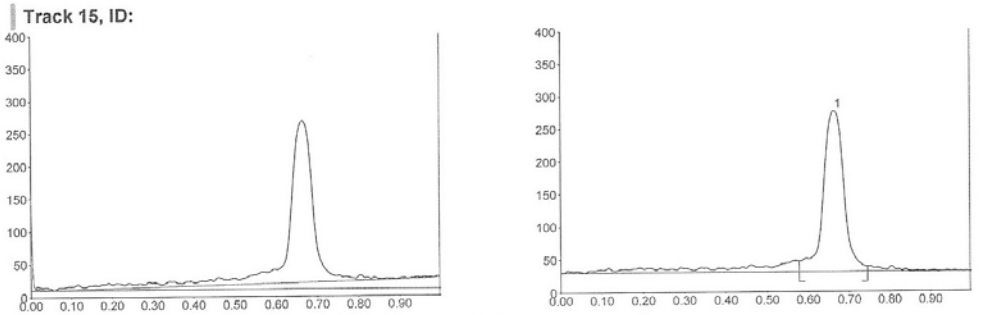
Figure 27 (Cont.). TLC-densitometry chromatogram (plate G – sample No. 12-15)



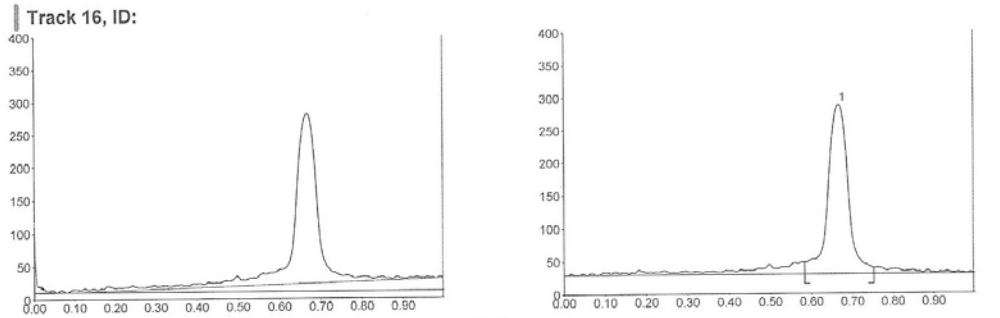
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.56	21.5	0.66	226.6	95.34	0.76	8.3	7664.2	97.78	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.27	3.1	0.30	13.7	5.22	0.33	4.6	159.8	2.06	unknown *
2	0.54	16.0	0.67	248.6	94.78	0.76	6.5	7599.6	97.94	unknown *

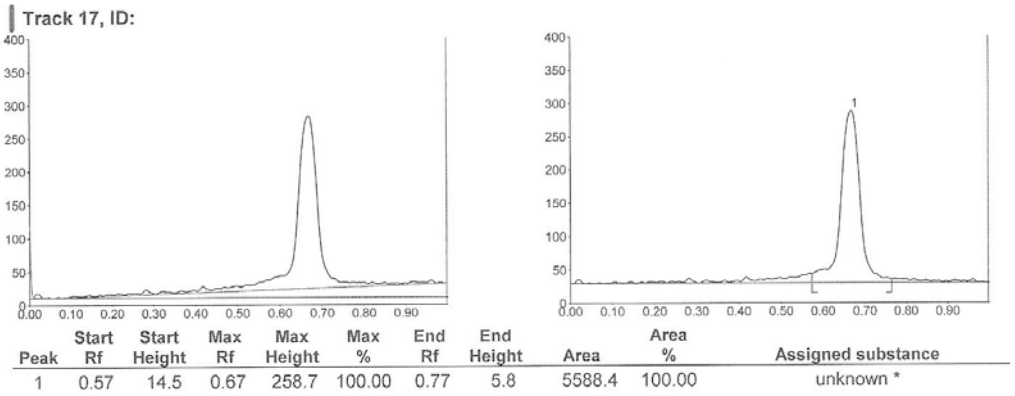


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	16.5	0.67	246.3	100.00	0.75	7.8	5784.8	100.00	unknown *



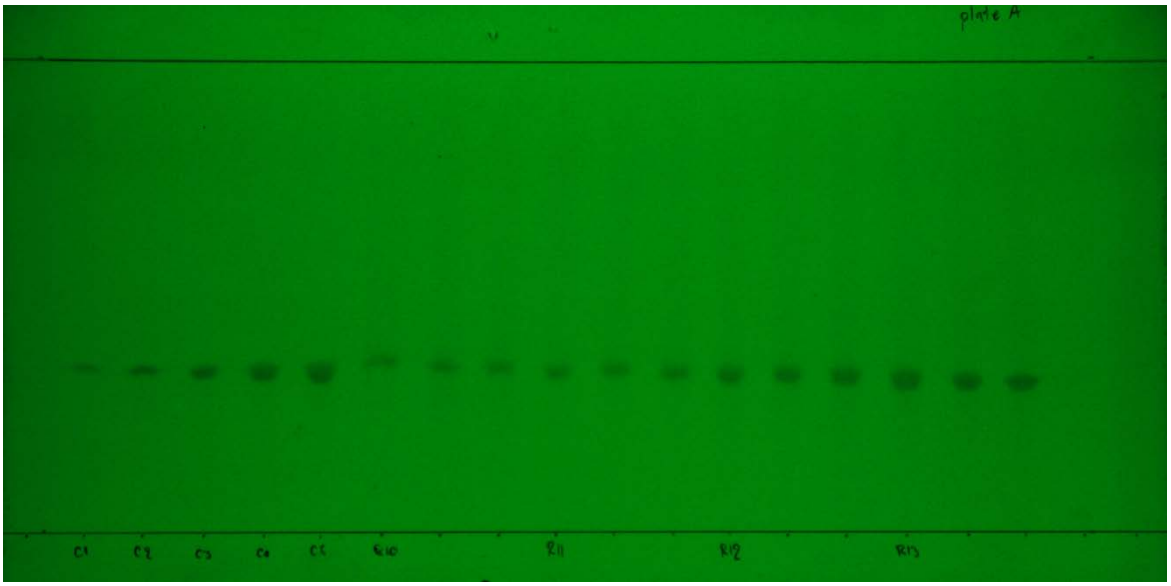
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.58	18.1	0.67	258.1	100.00	0.76	9.2	5912.3	100.00	unknown *

Figure 27 (Cont.). TLC-densitometry chromatogram (plate G – sample No. 12-15)

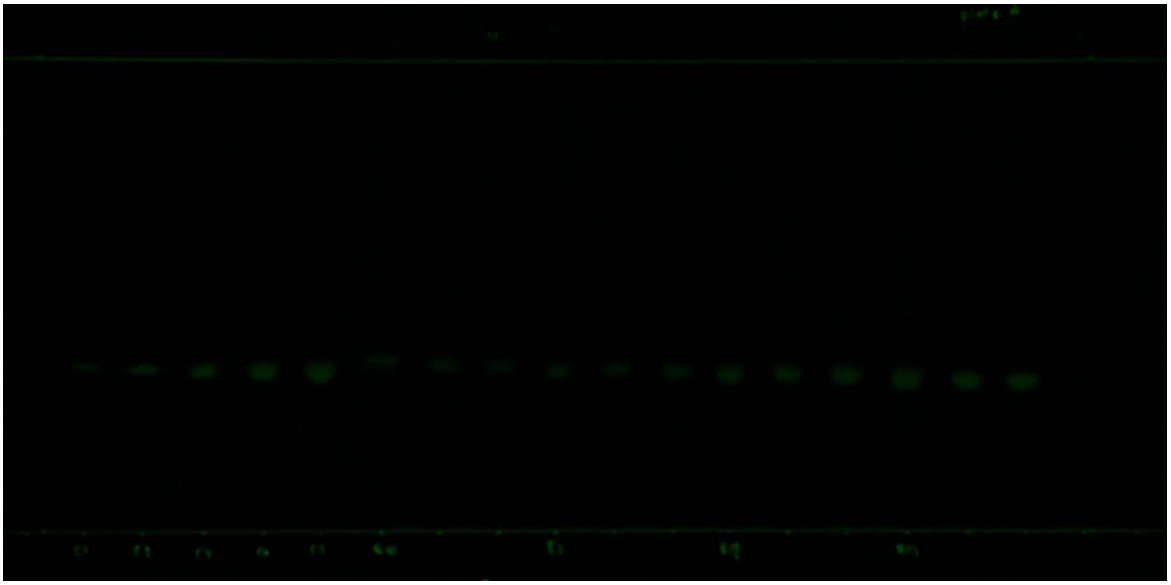


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.57	14.5	0.67	258.7	100.00	0.77	5.8	5588.4	100.00	unknown *

Figure 27 (Cont.). TLC-densitometry chromatogram (plate G – sample No. 12-15)

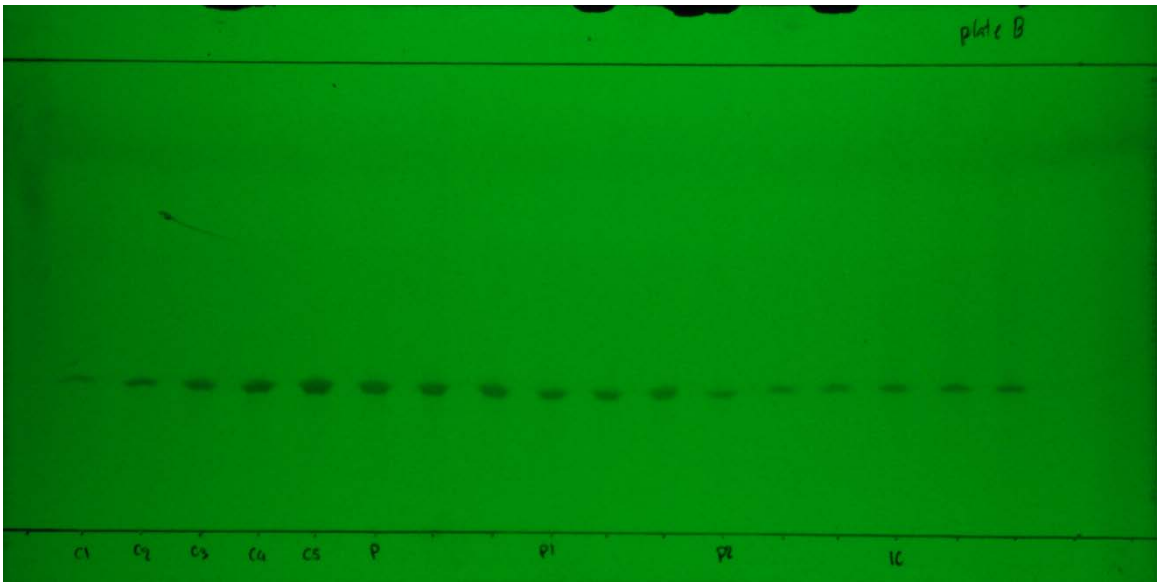


(A)

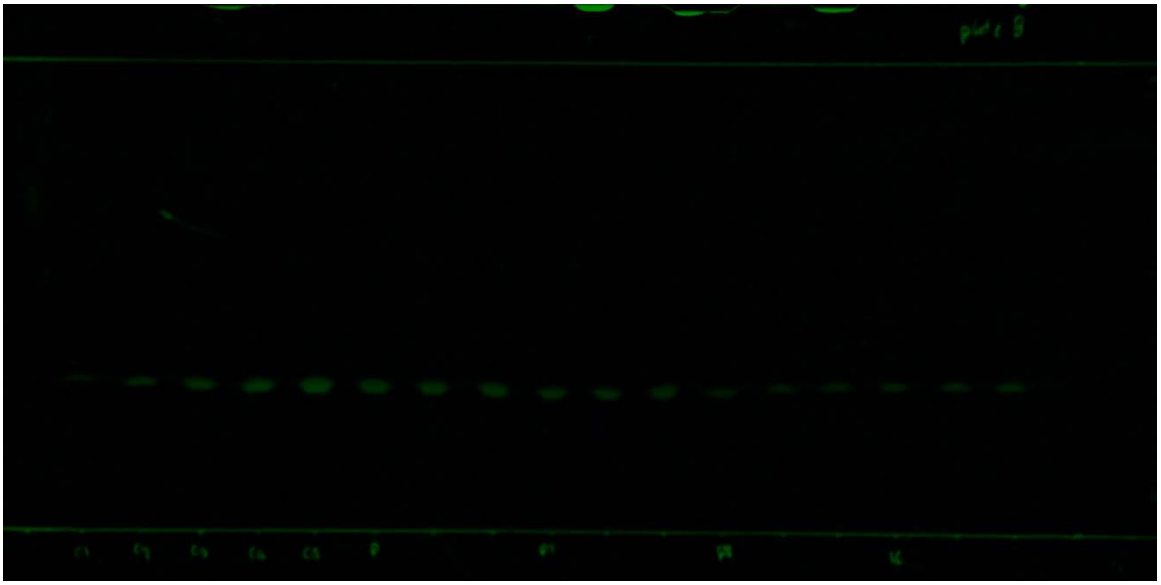


(B)

Figure 28. The TLC plates (plate A–Accuracy) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

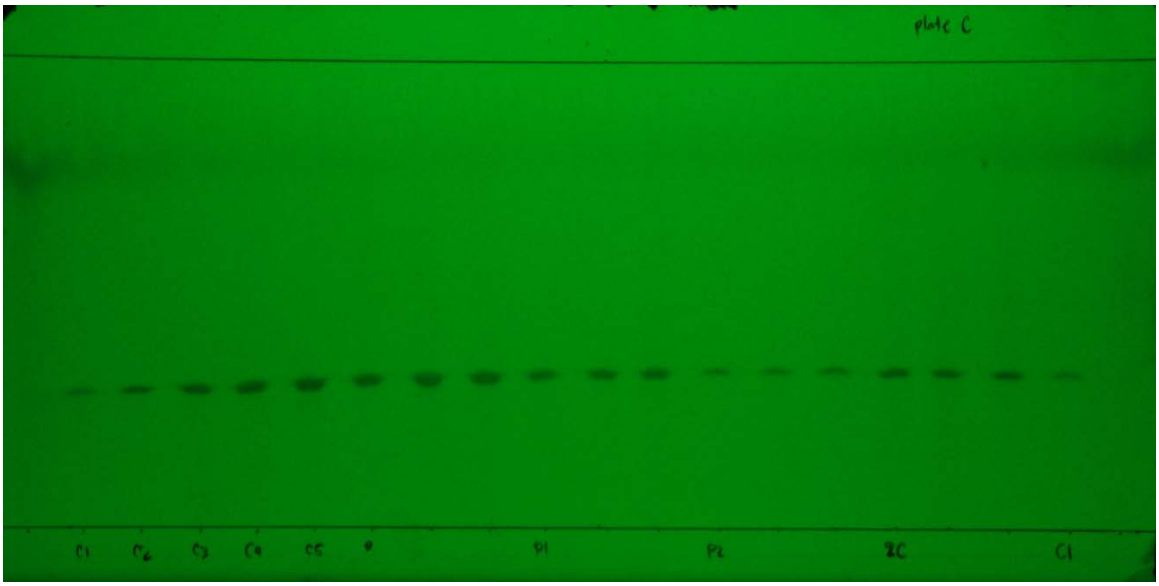


(A)

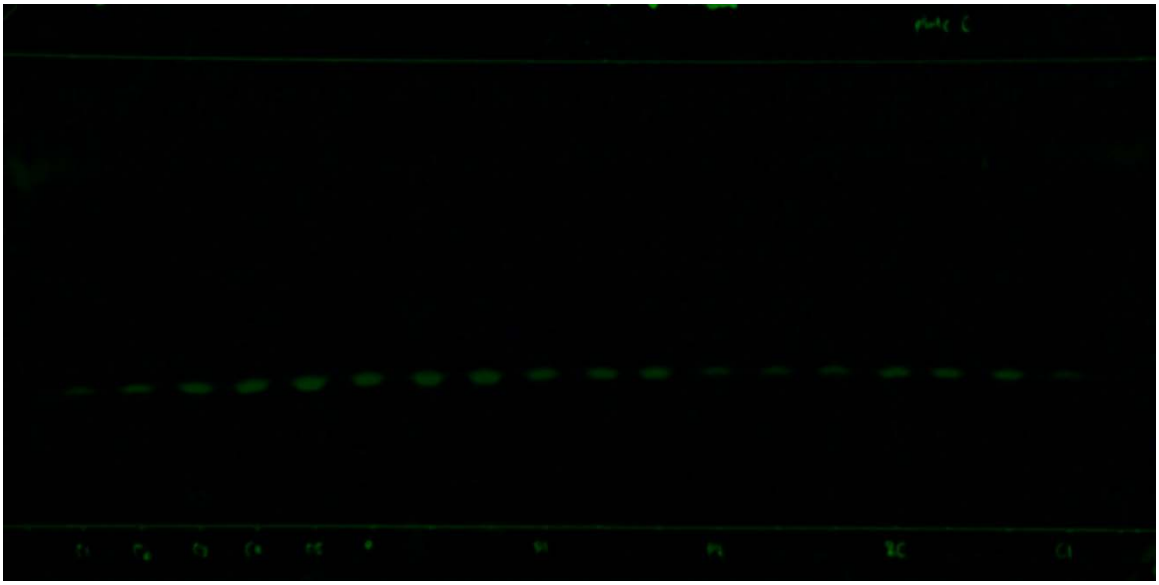


(B)

Figure 29. The TLC plates (plate B –Precision & sample No.1) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

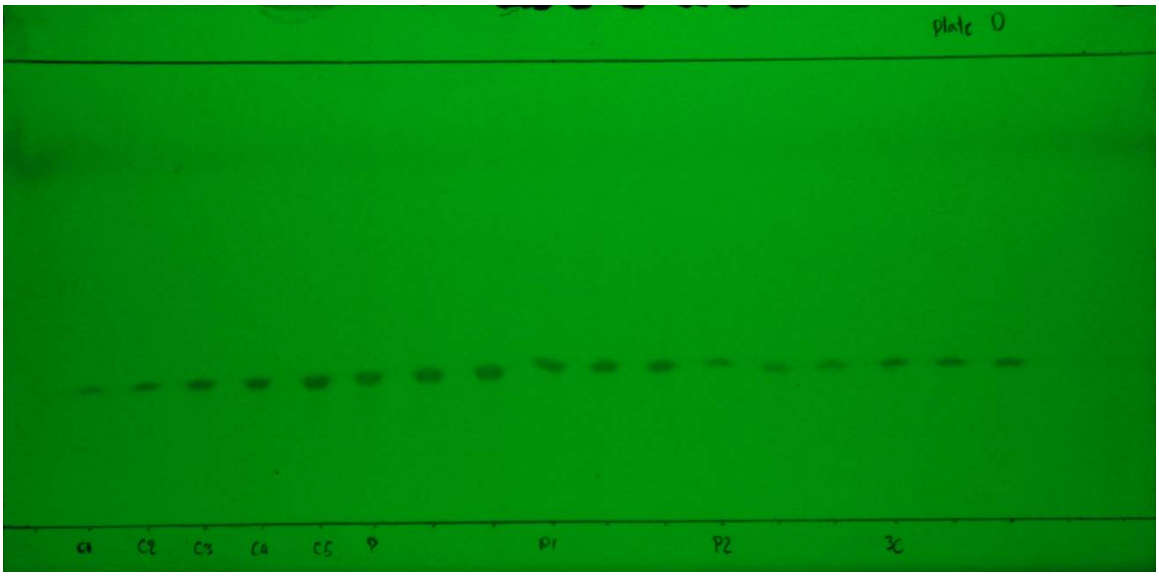


(A)

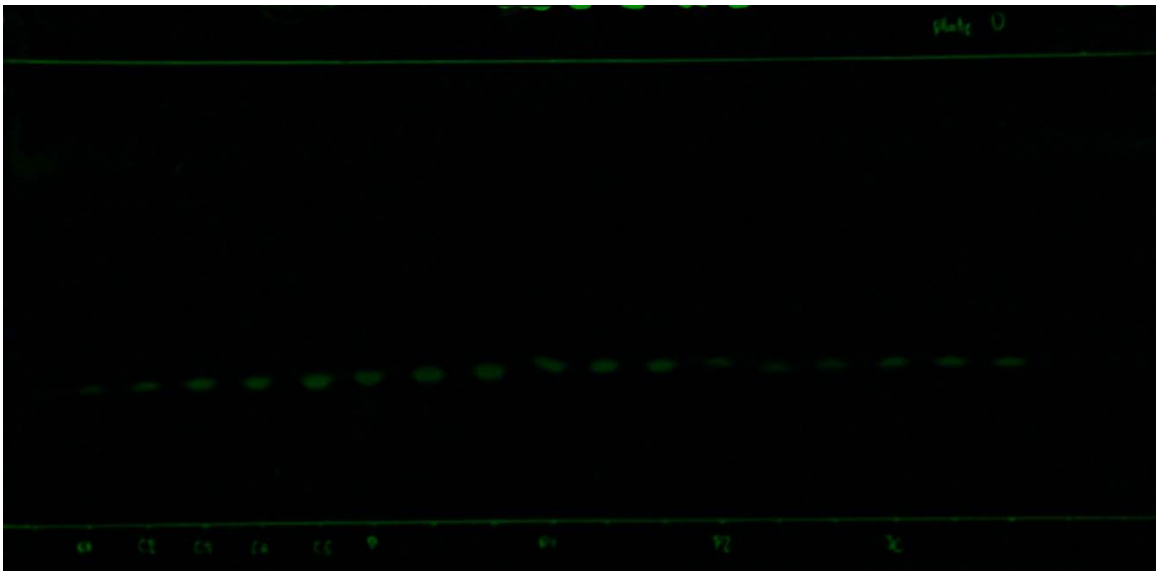


(B)

Figure 30. The TLC plates (plate C – Precision & sample No.2) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

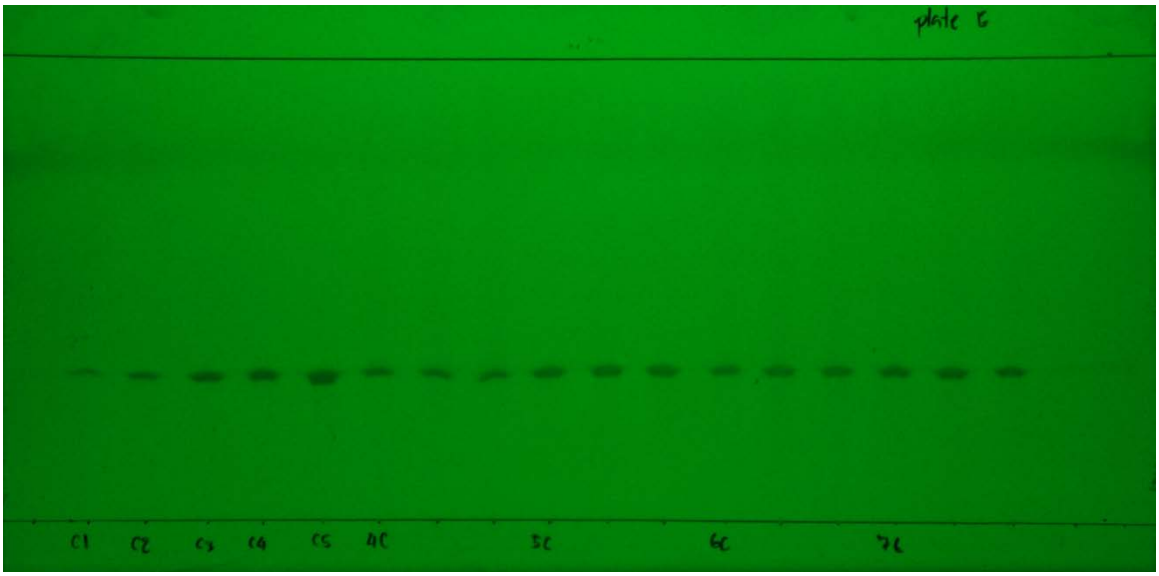


(A)

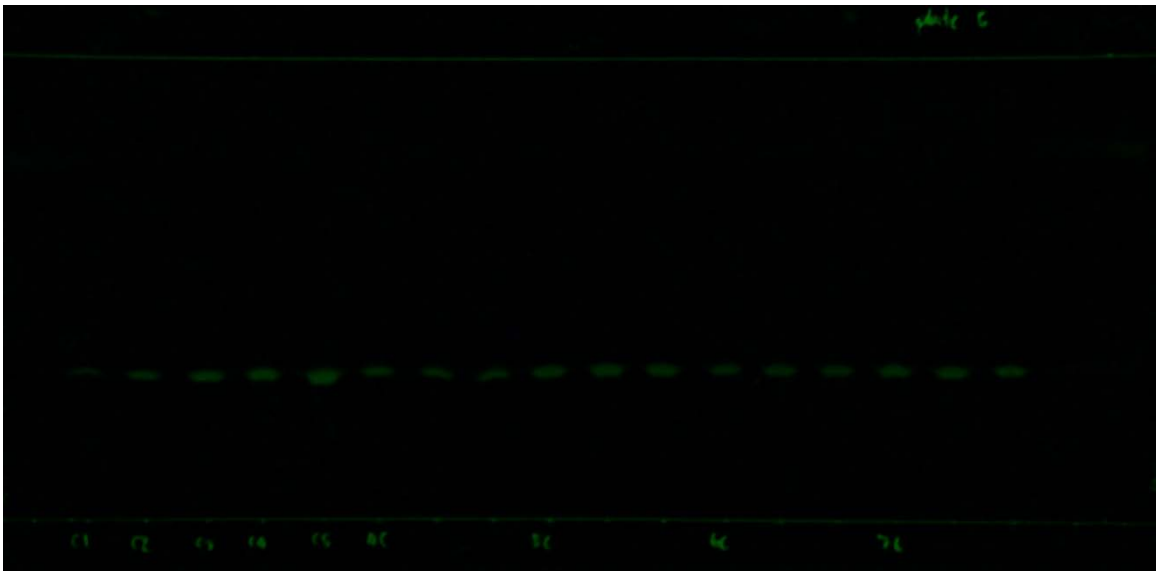


(B)

Figure 31. The TLC plates (plate D – Precision & sample No.3) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

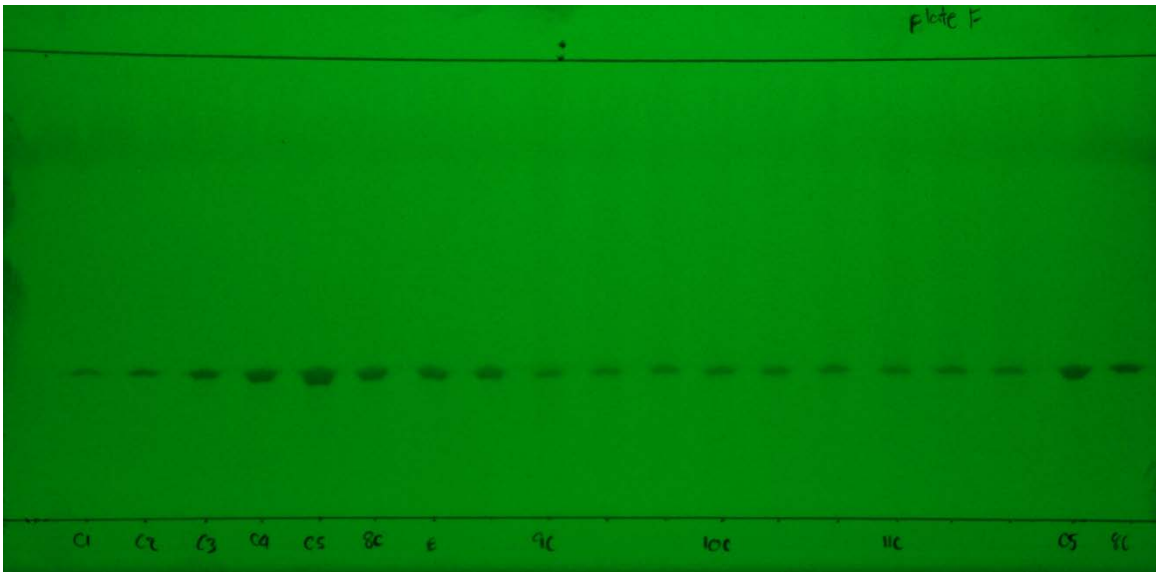


(A)

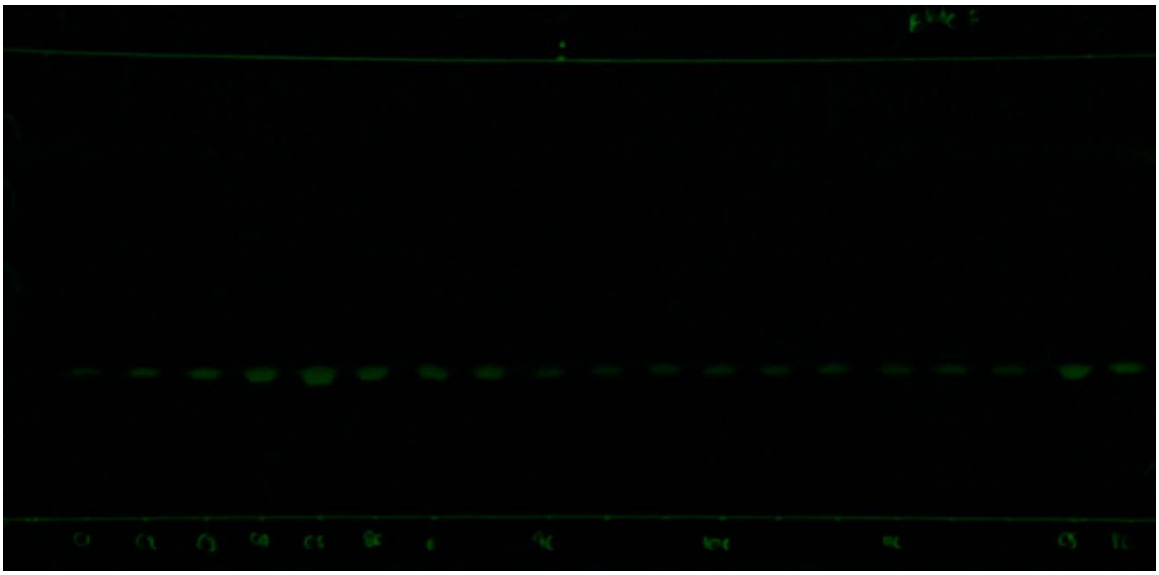


(B)

Figure 32. The TLC plates (plate E – sample No. 4-7) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

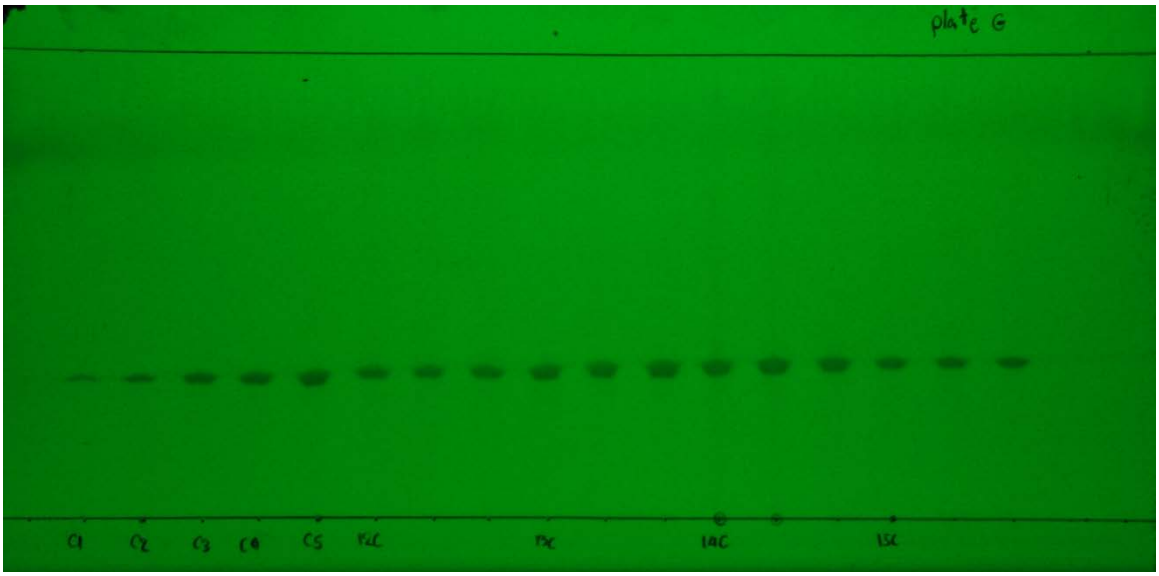


(A)

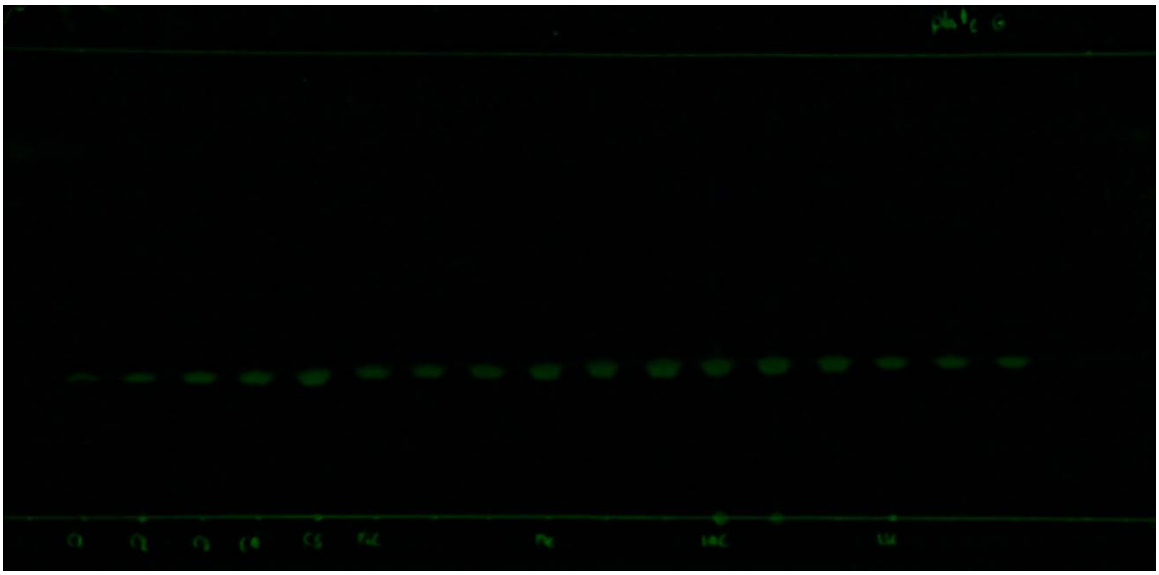


(B)

Figure 33. The TLC plates (plate F – sample No. 8-11) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)



(A)



(B)

Figure 34. The TLC plates (plate G – sample No. 12-15) developed with n-butanol, n-propanol, 4 N ammonia (1:7:2) visual under 254 nm original image (A), with subtrack background by ImageJ software (B)

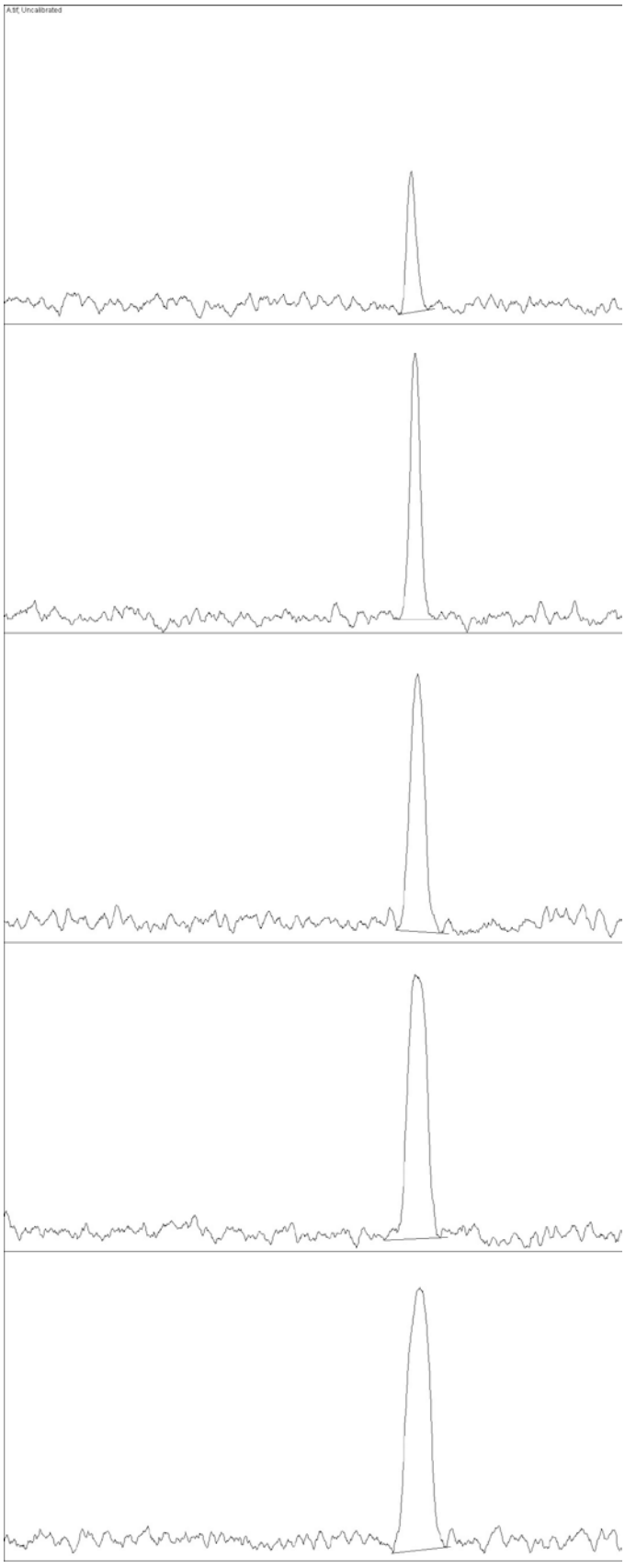


Figure 35. TLC-image analysis chromatogram (ImageJ software) (15 sources)

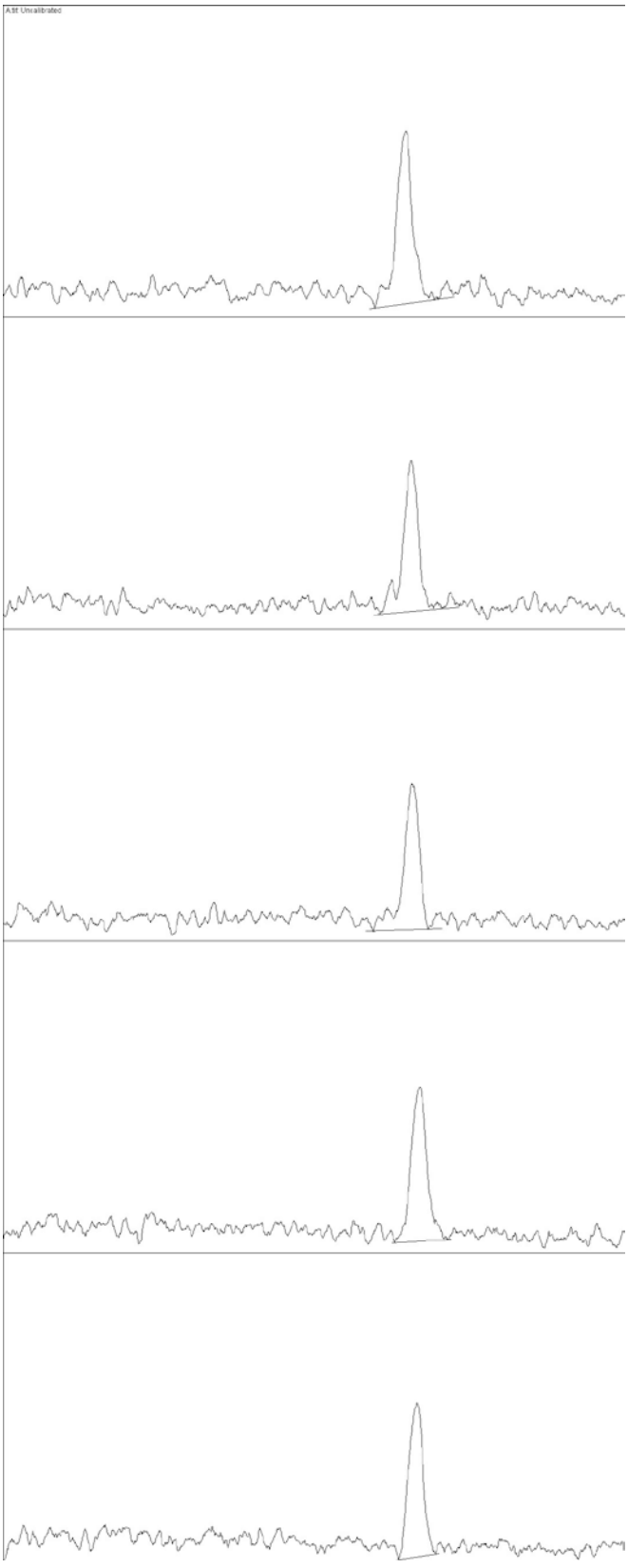


Figure 35 (cont.). TLC-image analysis chromatogram (ImageJ software) (15 sources)

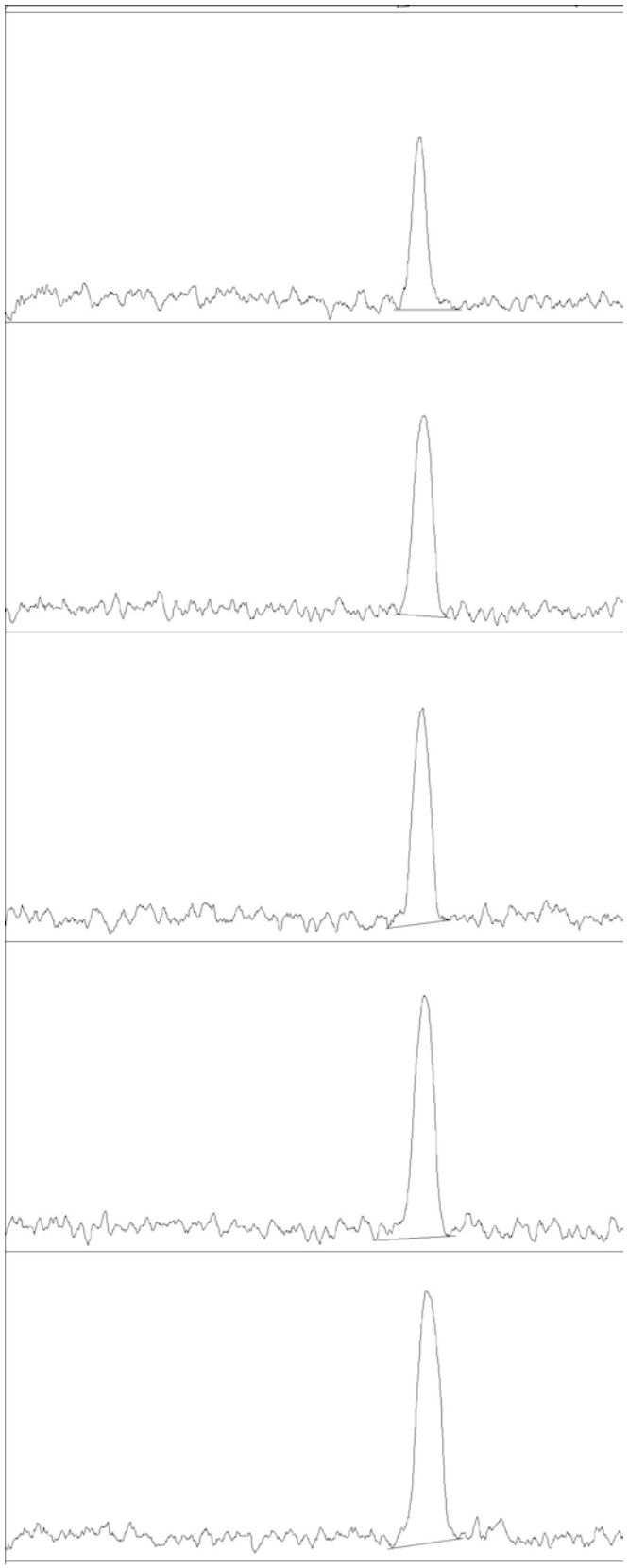


Figure 35 (cont.). TLC-image analysis chromatogram (ImageJ software) (15 sources)

VITAE

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Publication

Yukongphan, P., Palanuvej, C., and Ruangrungsi, N. The pharmacognostic specification of Ardisia elliptica fruits and their embelin content by TLC image analysis. Proceedings of the 38th Congress on Science and Technology of Thailand. Chiang Mai, 2012.