經濟部標準檢驗局台南分局 98 年度研究報告提要表			填表人：李和俊 填表日期：98.11.30
研究報告名稱	進口辣椒醬及辣味咖哩中蘇丹紅(1,2,3,4 號)含量之探討		
研究單位及人員	第三課 趙夢彩、李和俊	研究時間	自 98 年 1 月 1 日 至 98 年 12 月 31 日

報告內容提要

(一) 前言

蘇丹紅(Sudan red)是一種人工合成的紅色染料，常作爲工業染料，被廣泛用於如溶劑、油、蠟、汽油的增色以及鞋、地板等增光方面，禁用於食品、飼料，但目前已在食品中檢測到蘇丹紅染劑添加的情形。國際癌症研究機構(IARC)把蘇丹紅1號、2號、3號和4號列為第三類致癌物質，長期食用可能導致罹患癌症。本次專題研究選擇英國食品標準局FSA145A為檢驗方法，以無水乙醇萃取檢體後，以HPLC/UV分析。

(二) 材料與方法

本次所使用之檢體係購自台南分局轄區商店，進口辣椒醬 5 件，進口辣味咖哩 2 件，總共 7 件，原產地為馬來西亞 1 件，韓國 1 件，美國 1 件，泰國 3 件，日本 1 件。

(三) 結果與討論

1、蘇丹紅色素非法定食用人工色素，依法不得添加，本次 7 個檢體皆未檢出蘇丹紅色素，可安心食用。

2、本次檢驗之可檢下限蘇丹紅 1、2、3、4 號分別為:0.2 ppm、0.3 ppm、0.4 ppm、0.65 ppm。

3、使用英國食品標準局 FSA145A 所用之移動相配方，不需使用梯度沖提即可於 15 分鐘內分析完成，基線平坦且有效分離，為良好之移動相配方；但三乙胺為毒性化學物質，取用須注意通風及小心使用。

4、檢驗完成後應以沖提強度較移動相高之異丙醇等溶劑清洗，再以甲醇、水或不含鹽類之移動相清洗鹽類，後以約 50%甲醇做保存液避免管柱乾燥及長菌。

5、民眾在選購食品時，應向信譽良好的零售商和膳食供應商購買食物，注意標示所列的色素需為法定食用色素，避免選購標示不明、顏色異常鮮紅或售價過低的辣椒醬及辣味咖哩，並注意飲食均衡，減少因偏食導致過量攝入不良食品的機率。

目錄

(一)、前言

蘇丹紅(Sudan red)是一種人工合成的紅色染料，常作爲工業染料，被廣泛用於如溶劑、油、蠟、汽油的增色以及鞋、地板等增光方面，禁用於食品、飼料，但目前已在食品中檢測到四種蘇丹紅染劑的添加情形。國際癌症研究機構(IARC)把蘇丹紅1號、2號、3號和4號列為第三類致癌物質，長期食用可能導致罹患癌症。

2003年5月法國在一批自印度進口之辣椒產品中檢出有害色素蘇丹紅1號，歐盟執委會隨即公告禁止含有該色素之辣椒及其他產品輸入。由於蘇丹紅1號用後不容易褪色，可以彌補辣椒放置久後變色的現象，保持辣椒鮮亮的色澤。有些業者將玉米等植物粉末用蘇丹紅1號染色後，混在辣椒粉中，以降低成本牟取利益。中國曾發生「紅心鴨蛋」事件，不肖農夫將蘇丹紅4號加入飼料當中，由於飼料與蛋黃的顏色息息相關，蛋黃因易吸收脂溶性色素而顯的鮮豔。印度等一些國家在加工辣椒粉的過程亦常添加蘇丹紅色素。

蘇丹紅主要包括Ⅰ、Ⅱ、Ⅲ和Ⅳ四種類型:

一、 蘇丹紅I（Sudan I）

- 化學名稱：1-苯基偶氮-2-萘酚(1- phenylazo-2-naphthalenol）
- 分子式:$C_{16}H_{12}N_2O$

◆ 分子量：248.28

二、 蘇丹紅II（Sudan II）

◆ 化學名稱：1-[(2,4-二甲基苯)偶氮]-2-萘酚

(1-[(2,4-dimethylphenyl)azo]-2-naphthalenol）

◆ 分子式:$C_{18}H_{16}N_2O$

◆ 分子量：276.33

三、 蘇丹紅III（Sudan III）

◆ 化學名稱：1-{[4-(苯基偶氮)苯基] 偶氮}-2-萘酚

(1-{[4-(phenylazo)phenyl]azo]-2-naphthalenol）

◆ 分子式:$C_{22}H_{16}N_4O$

◆ 分子量：352.39

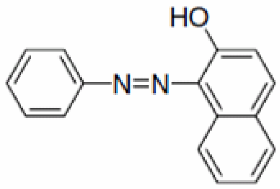
四、 蘇丹紅IV（Sudan IV）

◆ 化學名稱：1-{{2-甲基-4-[(2-甲基苯)偶氮]苯基}偶氮}-2-萘酚

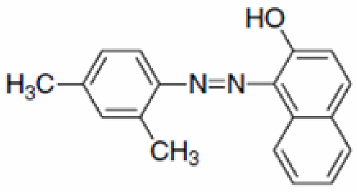
(1-{{2-methyl-4-[(2-methylphenyl)azo]phenyl}azo]-2-naphthalenol）

◆ 分子式:$C_{24}H_{20}N_4O$

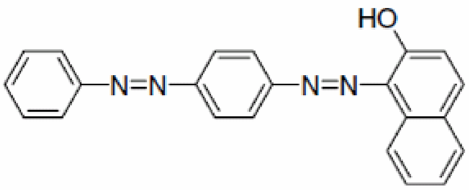
- 分子量：380.44



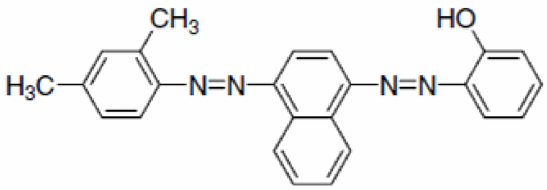
Sudan I (1-[(2,4-dimethylphenyl)azo]-2-naphthalenol)



Sudan II (1-(phenylazo)-2-naphthol)



SudanIII (1-(4phenylazophenylazo)-2-naphthol)



Sudan IV (o-tolyazo-o-tolylazo-betanaphthol)

目前本國公告之相關檢驗法僅有中華民國國家標準CNS10889-食品中色素之檢驗法，先以有機溶劑進行初步分離，萃取脂溶性色素於乙醚層，再以薄層層析(TLC)進行鑑別試驗。而中國國家質量監督檢驗檢疫總局GB/T19681-2005食品中蘇丹紅染料的檢測方法高效液相色譜法，使用固相萃取管柱純化後，再以HPLC分析。查閱文獻中檢驗方法以HPLC/UV為主，而其他檢驗方法亦在開發中，例如GC/MS、LC/MS/MS、HPLC/螢光和ELISA抗體法等。本次專題研究選擇英國食品標準局FSA145A為檢驗方法，以無水乙醇萃取檢體後，以HPLC/UV分析。

（二）、材料與方法

一、檢體來源

本研究所使用之檢體係購自台南分局轄區商店，進口辣椒醬5件，進口辣味咖哩2件，總共7件，原產地為馬來西亞1件，韓國1件，美國1件，泰國3件，日本1件。

二、試藥

蘇丹紅色素皆購自美國Sigma公司（Fluka），蘇丹1號product number:51383(analytical standard，純度98.3%)，蘇丹2號product number:07937(analytical standard，純度99.3% ），蘇丹3號product number:68562(analytical standard，純度96.0% ），蘇丹4號product number:67386(analytical standard，純度96.2%)。溶劑與藥品均為LC級或試藥特級。

三、儀器設備

高效液相層析儀（High Performance Liquid Chromatography，HPLC)：Agilent，主機HP-1100，記錄器HP-2300，配備紫外光/可見光檢測器(UV/VIS)。

四、移動相的配製

取3.850g之醋酸銨溶於1公升甲醇並混合均匀，配製成

0.05mole/L 之醋酸銨/甲醇溶液，將此醋酸銨/甲醇溶液與乙腈以 1:4 比例混合後，每公升再加入 1 毫升三乙胺。

五、標準品儲存溶液之配製

將購得之 4 種蘇丹色素對照標準品(規格 25 mg)，置入 100 mL 之定量瓶中，用乙酸乙酯清洗剩餘之標準品粉末，將洗液收集至定量瓶中，加乙酸乙酯至約 90 ml，以超音波振盪至完全溶解，於 25℃溫度以乙酸乙酯定容至 100 mL，配製成約 250ppm 之標準品，避光低溫貯藏。

六、檢液調製與分析

樣品混合均勻後，取 5 克樣品加入 20 ml 無水乙醇(純度 99% 以上)，高速震盪 3 分鐘，離心 10 分鐘，取上清液以 0.45 μm 過濾子過濾後避光低溫貯存。

七、HPLC 分析條件

1. 層析管柱：Inertsil ODS-3v， 5 µm ，RP-C18，
   LotNo.VQ5-2491，4.6 × 250 mm，GL Sciences 公司。
2. 移動相溶液：沖洗平衡約 1 小時，至基線穩定再進行樣品分析。
3. 流速:1.3 ml/mins。
4. 檢測波長：UV 檢出器設定 476 nm。

5. 注射量：10 μL。

6. 管柱溫度：25 ℃。

7. 偵測時間：12 分鐘。

八、可檢下限之測定

將標準品稀釋到訊號與基線相比，可維持波峰無分岔且波形完整之最低濃度，再乘以5倍稀釋倍數估算。

九、檢量線之製作

將標準品混合溶液，以移動相做一系列之稀釋，配製五種濃度 0.5 ~ 6.25 ppm 蘇丹紅標準品溶液，過濾後進行 HPLC 之分析。由波峰面積與濃度，計算蘇丹紅色素 1 至 4 號檢量線，並求得線性迴歸方程式及相關係數。

十、檢體濃度換算

檢體濃度(ppm) = $\frac{\mathrm{A}}{\mathrm{B}} \times Y \times \frac{\mathrm{S}}{\mathrm{S}+20}$

A:檢體訊號面積

B:標準品訊號面積

Y:標準品濃度

S:樣品重量

（三）、結果

一、標準品儲存溶液之配製

本次所購得之蘇丹紅標準品1至4號純度依序為98.3%、99.3%、96.0%、96.2%，將250ppm標準品溶液換算純度後，蘇丹紅1至4號各別濃度為245.8 ppm、248.3 ppm、240.0 ppm、240.5 ppm，

二、不同移動相流速的比較

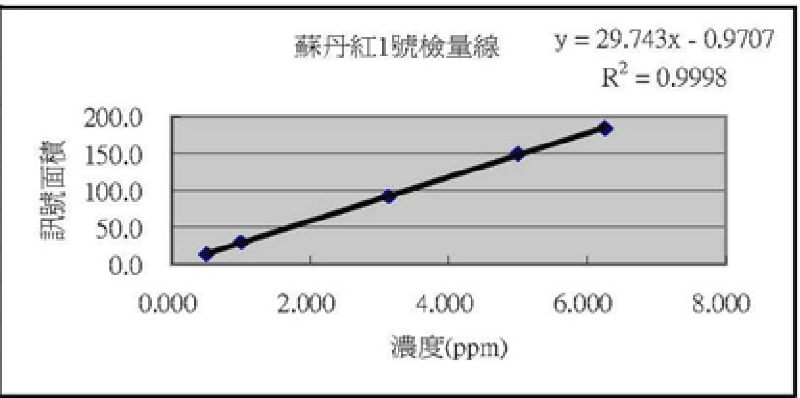
流速為1.00 ml/mins時有峰脫尾的情形，故以加快流速改善，並可減短分析的時間，而考量儀器的狀況後，本次選擇了1.3 ml/mins作為流速，全部分析時間約可在12分鐘以內完成。

流速	滯留時間RT(mins)			
(ml/mins)	蘇丹紅1號	蘇丹紅2號	蘇丹紅3號	蘇丹紅4號
1.00	4.590	7.695	9.373	13.802
1.25	4.462	6.141	7.474	10.995
1.30	4.306	5.925	7.214	10.609
1.50	3.723	5.122	6.234	9.158

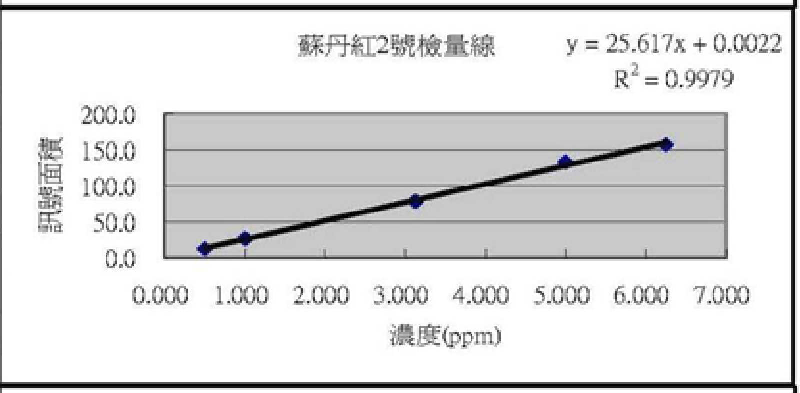
三、標準品檢量線

製作0.5ppm、1.0ppm、3.125ppm、5.0ppm、6.25ppm共5點標準品檢量線，以濃度(ppm)為X座標，訊號峰面積為Y座標。

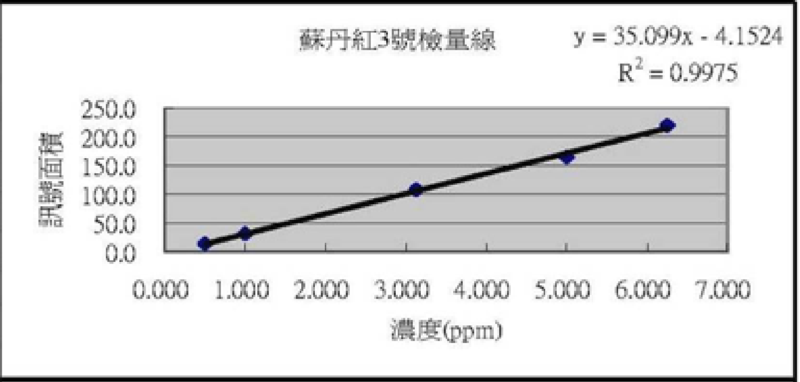
蘇丹紅1號檢量線	
濃度(x)	訊號面積（y）
0.5	13.25433
1	29.39262
3.125	91.52479
5	149.23882
6.25	183.90424
r =	0.9998
m =	29.743
b =	-0.9707



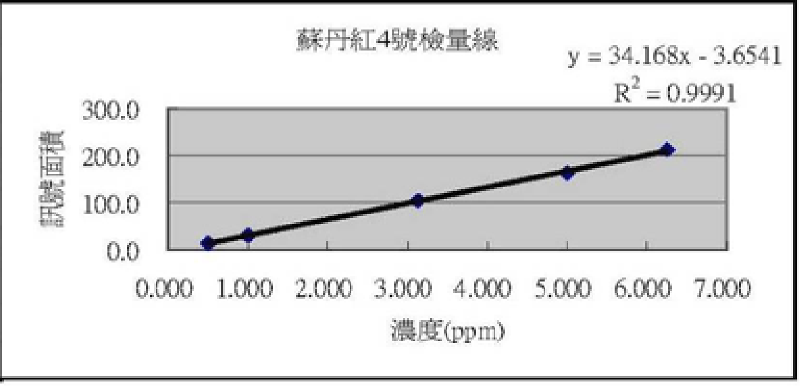
蘇丹紅2號檢量線	
濃度(x)	訊號面積（y）
0.5	12.15268
1	26.23149
3.125	78.35619
5	132.7957
6.25	157.14949
r =	0.9979
m =	25.617
b =	0.0022



蘇丹紅3號檢量線	
濃度(x)	訊號面積（y）
0.5	13.66205
1	31.01055
3.125	107.79172
5	164.20773
6.25	219.76424
r =	0.9975
m =	35.099
b =	-4.1524



蘇丹紅4號檢量線	
濃度(x)	訊號面積（y）
0.5	13.59368
1	30.73795
3.125	104.08639
5	163.07317
6.25	212.65176
r =	0.9991
m =	34.168
b =	-3.6541



四、可檢下限計算

四種蘇丹紅色素的可檢下限在0.04 – 0.13 ppm之間，當稀釋10倍時為為0.4-1.3 ppm，當稀釋5倍時為0.2-0.65 ppm。

ppm	蘇丹紅1號	蘇丹紅2號	蘇丹紅3號	蘇丹紅4號
可檢下限(樣品未稀釋時)	0.04	0.06	0.08	0.13
可檢下限(乘以10倍)	0.4	0.6	0.8	1.3
可檢下限(乘以5倍)	0.2	0.3	0.4	0.65

五、檢體資料及檢驗結果

本次 7 個檢體皆未檢出蘇丹紅色素。(ND=未檢出)

編號	類別	品名	檢體重量(g)	檢體標示		蘇丹紅含量(ppm)			
				原產地	有效期限	1	2	3	4
1	辣椒醬	泰式酸辣醬	5.04	馬來西亞	27/08/2010	ND	ND	ND	ND
2	辣椒醬	辣椒醬	5.01	韓國	10/08/2010	ND	ND	ND	ND
3	辣椒醬	紅椒汁	5.00	美國	16/12/2013	ND	ND	ND	ND
4	辣椒醬	辣椒醬	4.99	泰國	09/11/2010	ND	ND	ND	ND
5	辣椒醬	辣椒膏	5.02	泰國	17/12/2009	ND	ND	ND	ND
6	咖哩醬	紅咖哩醬	5.03	泰國	20/06/2011	ND	ND	ND	ND
7	咖哩塊	辣味咖哩	5.02	日本	29/10/2010	ND	ND	ND	ND

(四)、討論

1、 檢量線於0.5-6ppm有良好線性，四種蘇丹紅色素檢量線相關係數R值分別為0.9998、0.9979、0.9975和0.9991，R值達到0.9975以上，儀器再線性佳，故可使用單一濃度標準品比對樣品即可。

2、 蘇丹紅色素非法定食用人工色素，依法不得添加，本次7個檢體皆未檢出蘇丹紅色素，可安心食用。

3、 樣品前處理稀釋10倍時，蘇丹紅4號可檢下限為1.3 ppm較差，本次選擇稀釋5倍，使蘇丹紅4號可檢下限為0.65 ppm，故本次檢驗之可檢下限蘇丹紅1、2、3、4號分別為:0.2 ppm、0.3 ppm、0.4 ppm、0.65 ppm。

4、 編號1號及2號檢體以乙醇萃取時，萃取液中具有成團狀現象，經比對其所標示的成分，在7個檢體中只有此2個檢體有添加澱粉，可能為澱粉與乙醇不互融而結成團狀。

5、 使用英國食品標準局FSA145A所用之移動相配方，不需使用梯度沖提即可於15分鐘內分析完成，基線平坦且有效分離，與其他檢驗方法相比是為良好之移動相配方；但三乙胺為毒性化學物質，取用須注意通風及小心使用。

6、 檢驗完成後C18管柱之清洗重要，由於樣品前處理未經過固相萃取管柱純化，因此樣品中含有之天然色素、胺基酸等各種小分子

成分，這些未知且複雜的成分可能殘留在管柱中，故檢驗完成後應以沖提強度較移動相高之異丙醇等溶劑清洗，再以甲醇、水或不含鹽類之移動相清洗鹽類，後以約50%甲醇做保存液避免管柱乾燥及長菌。

7、 民眾在選購食品時，應向信譽良好的零售商和膳食供應商購買食物，注意標示所列的色素需為法定食用色素，避免選購標示不明、顏色異常鮮紅或售價過低的辣椒醬及辣味咖哩，並注意飲食均衡，減少因偏食導致過量攝入不良食品的機率。

（六）、參考資料

1. 中華民國國家標準 CNS10889 食品中色素之檢驗法。
2. 中國國家質量監督檢驗檢疫總局 GB/T19681-2005:食品中蘇丹紅染料的檢測方法高效液相色譜法。
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5. 英國食品標準局(FOOD STANDARDS AGENCY)FSA145A 檢驗法。
6. 香港特別行政區政府食物安全中心。風險簡訊:食物中的蘇丹紅。
7. 吳白玟，張文亮，潘志寬，周薰修。食用油脂中蘇丹色素之分析。藥物食品檢驗局調查研究年報 25，302-307，2007。

(七)、附件

一、市購檢體照片

1 號檢體:

2 號檢體:

3 號檢體:


TABASCO
品　名：TABASCO 紅椒汁
3
3


TABASCO® pepper sauce
McILHENNY CO.
TABASCO BRAND PEPPER SAUCE
60 ml
PRODUCT OF THE U.S.A.
SHAKE WELL
TABASCO® is also available in the following flavours
2007/01/22

4 號檢體:


HOT
GOLDEN M
HOT CHILLI
4
4


8 851954 103918
TIS 242-990
南洋美味，盡在寶萃
寶萃南洋美食有限公司
台北市內湖區港華街3號1樓
台北：02-2797-7118　台中：04-2317-7070
SRIRACHA CHILLI SAUCE "GOLDEN MOUNTAIN"
品　　名：金山是拉差辣椒醬
成　　份：辣椒，糖，蒜頭，醋，鹽，
不含防腐劑及色素
內 容 量：230 公克
有效日期：標於標紙（標示次序為西元日月年）
(JAN/1月,FEB/2月,MAR/3月,APR/4月,MAY/5月,JUN/6月,
JUL/7月,AUG/8月,SEP/9月,OCT/10月,NOV/11月,DEC/12月)
出 口 商：THAI WORLD IMPORT & EXPORT CO., LTD.

5 號檢體:


CHILLI PASTE ON SOY BEAN OIL
PAO) "MAESRI"
品 名：辣椒膏
內 容 量：450 公克
5
5
73-1-03826-1-0010
PRODUCT OF THAILAND


BRAND
MAESRI
น้ำพริกเผาชนิดปรุงอาหาร
CHILLI PASTE IN SOY BEAN OIL
(NAMPRIK PAO)
5
5

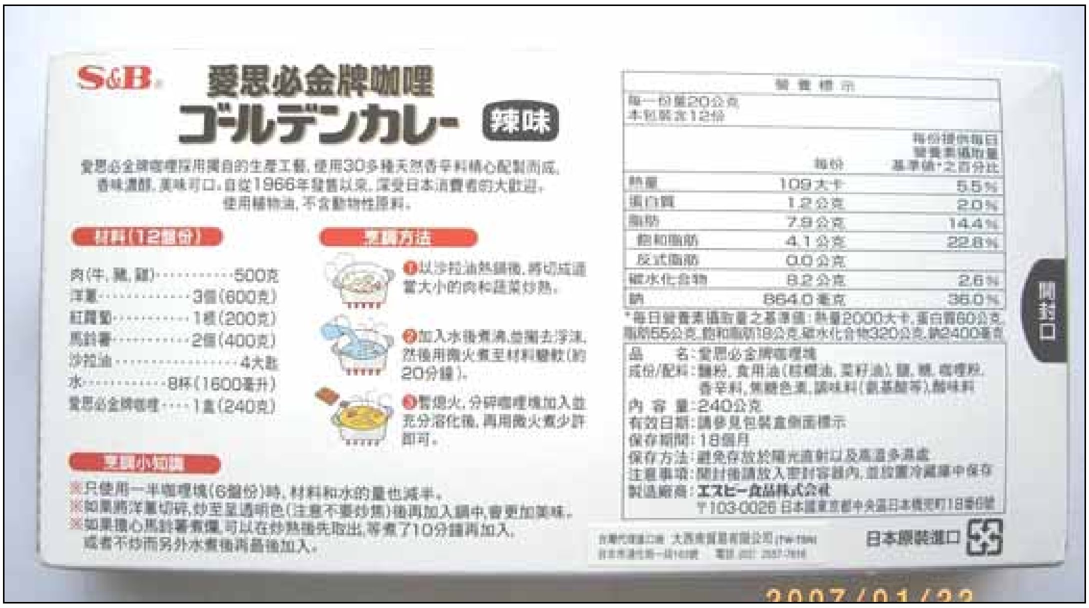
6 號檢體:


RED CURRY PASTE
品 名：紅咖哩醬
成 份：紅辣椒、蒜、薑、香茅、南薑、蝦膏、鹽、香料
淨 重：400公克
製 造 商：CURRY & SPICES CO.,LTD.
地 址：8/34 MOO 8 PETCHKASAEM RD., BANGKOK, THAILAND
進 口 商：尚泰豐有限公司
地 址：(105)台北市松山區延吉街9巷12之1號1樓
服務電話：(02)2570-1086
原 產 地：泰國
有效日期：標示於包裝上（日、月、西元年）
EXPIRY DATE
營養標示
6
6

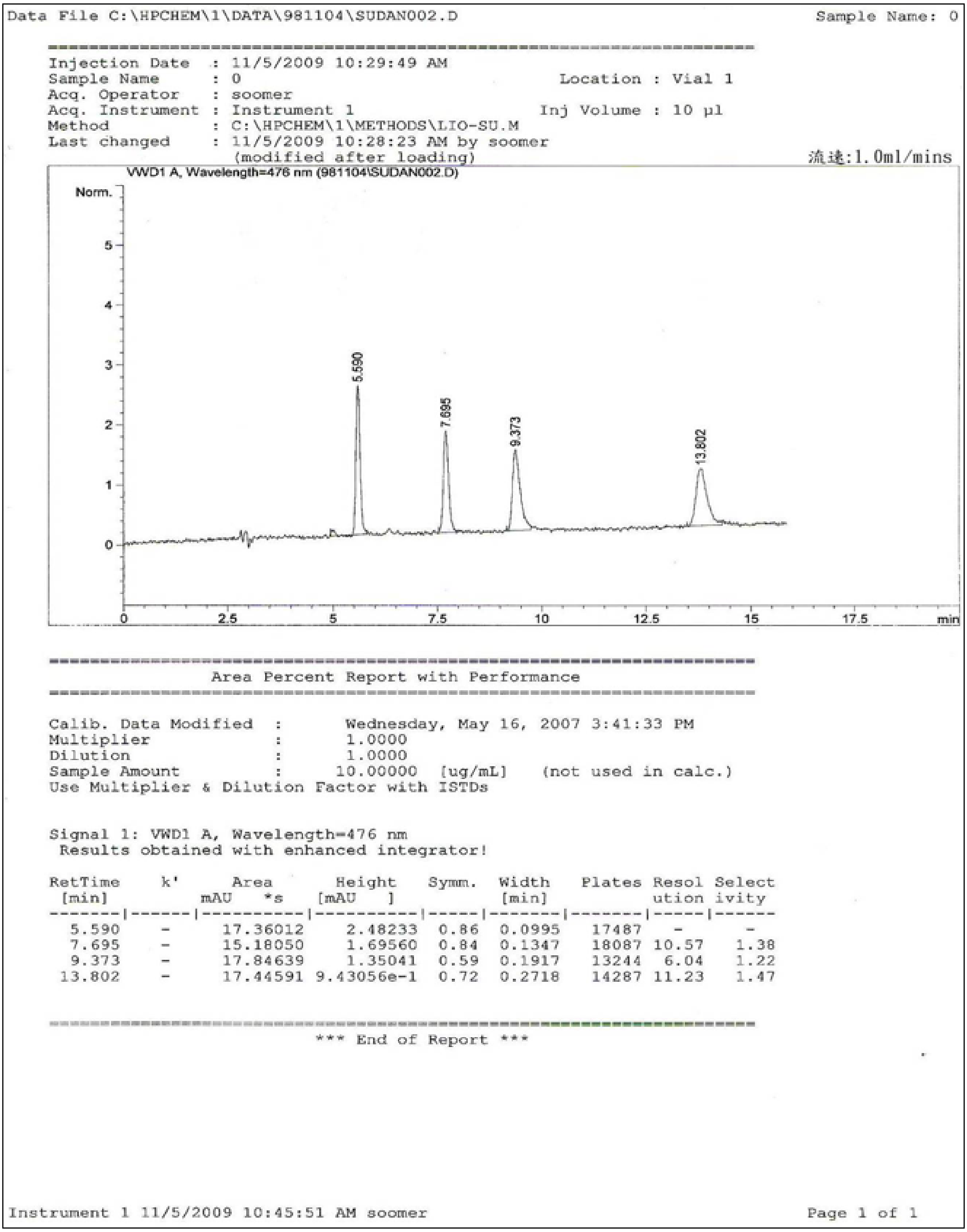

意
NAMJAI
น้ำใจ
RED CURRY PASTE
400 g
แกงเผ็ด
SERVING SUGGESTION
6
6
Product of Thailand

7 號檢體:


S&B
愛思必金牌咖哩
ゴールデンカレー
GOLDEN CURRY
香味豊かなキレのある味わい
日本原裝進口　不含動物性原料
240g
7
7
1234 5 HOT
辣味 12盤份


S&B
愛思必金牌咖哩
ゴールデンカレー
辣味
材料(12盤份)
烹調方法
烹調小知識
營養標示
開封口
日本原裝進口
2007/01/22

二、不同流速層析圖



```
Data File C:\HPCHEM\1\DATA\981104\SUDAN002.D                                Sample Name: 0

    =================================================================
    Injection Date  : 11/5/2009 10:29:49 AM
    Sample Name     : 0                              Location : Vial 1
    Acq. Operator   : soomer
    Acq. Instrument : Instrument 1                 Inj Volume : 10 µl
    Method          : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/5/2009 10:28:23 AM by soomer
                      (modified after loading)
```

流速:1.0ml/mins

```
    =================================================================
                   Area Percent Report with Performance
    =================================================================

    Calib. Data Modified  :       Wednesday, May 16, 2007 3:41:33 PM
    Multiplier            :       1.0000
    Dilution              :       1.0000
    Sample Amount         :      10.00000  [ug/mL]   (not used in calc.)
    Use Multiplier & Dilution Factor with ISTDs

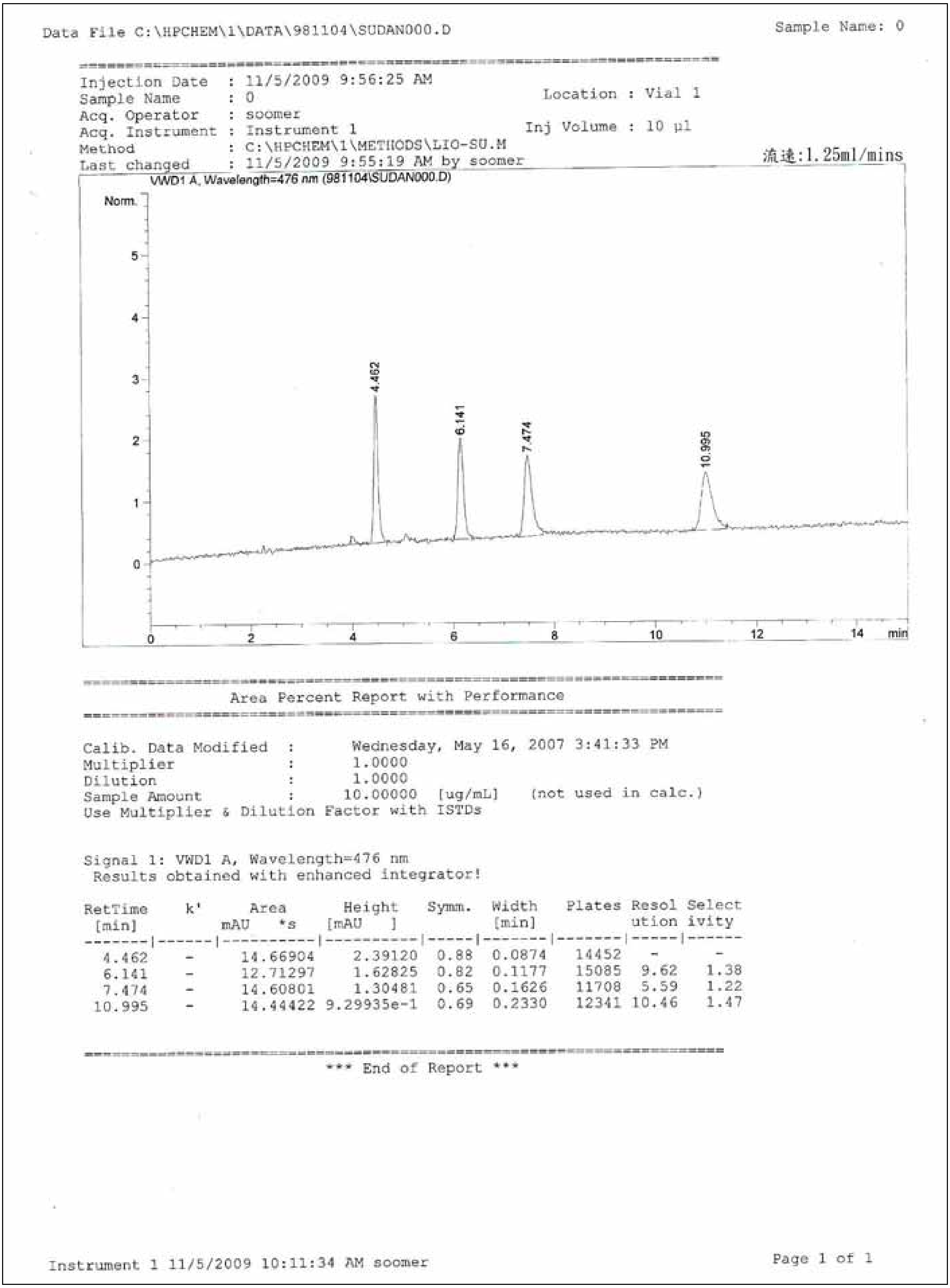

    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'      Area       Height    Symm.  Width    Plates Resol Select
     [min]           mAU   *s   [mAU    ]          [min]           ution ivity
    -------|------|-----------|-----------|-----|-------|-------|-----|------
      5.590   -      17.36012    2.48233  0.86  0.0995   17487   -      -
      7.695   -      15.18050    1.69560  0.84  0.1347   18087 10.57   1.38
      9.373   -      17.84639    1.35041  0.59  0.1917   13244  6.04   1.22
     13.802   -      17.44591 9.43056e-1  0.72  0.2718   14287 11.23   1.47

    =================================================================
                           *** End of Report ***




Instrument 1 11/5/2009 10:45:51 AM soomer                               Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\981104\SUDAN000.D                          Sample Name: 0

     =================================================================
     Injection Date  : 11/5/2009 9:56:25 AM
     Sample Name     : 0                             Location : Vial 1
     Acq. Operator   : soomer
     Acq. Instrument : Instrument 1                Inj Volume : 10 µl
     Method          : C:\HPCHEM\1\METHODS\LIO-SU.M
     Last changed    : 11/5/2009 9:55:19 AM by soomer                 流速:1.25ml/mins
```

```
     =================================================================
                     Area Percent Report with Performance
     =================================================================

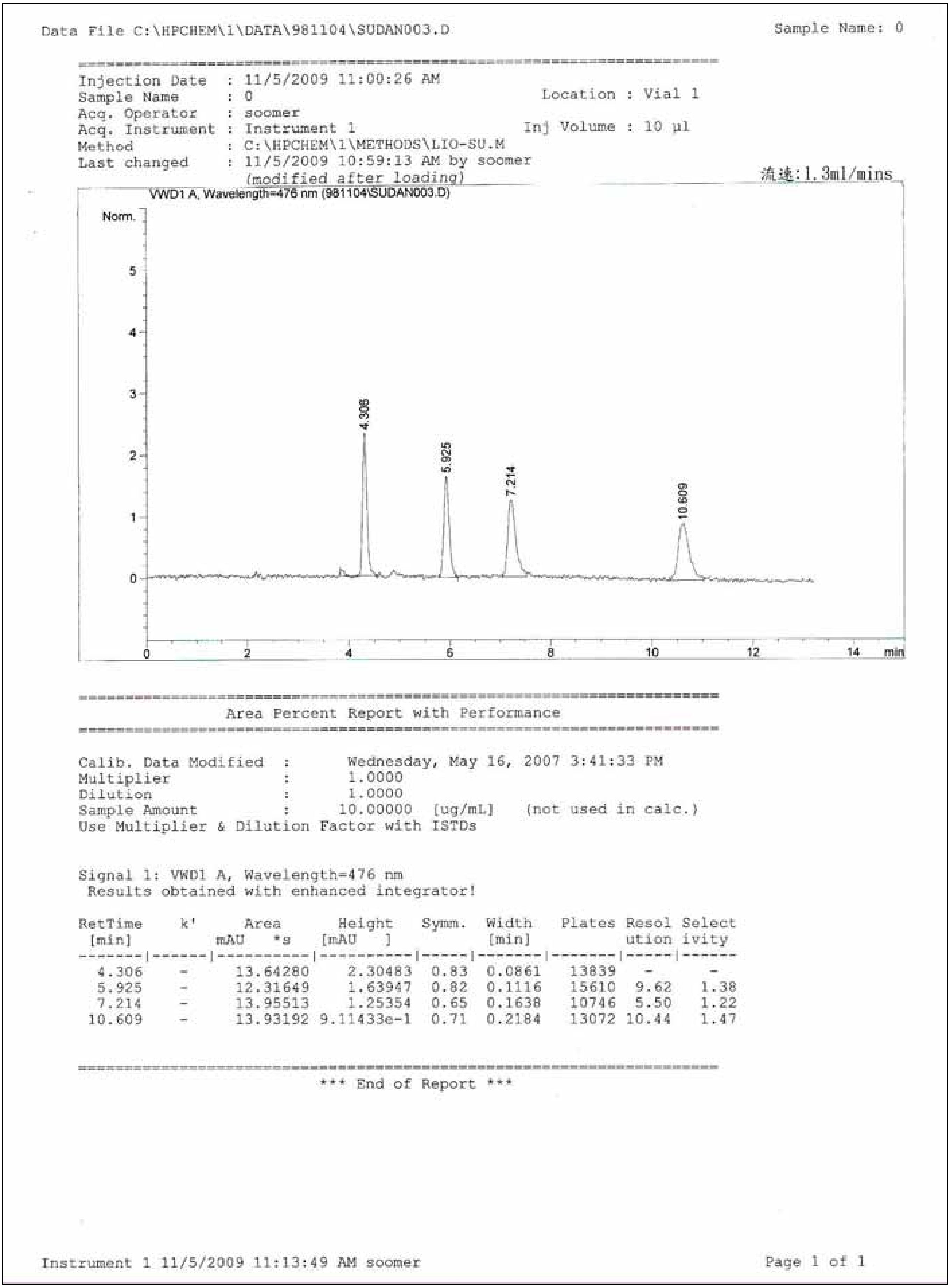
     Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
     Multiplier            :      1.0000
     Dilution              :      1.0000
     Sample Amount         :     10.00000  [ug/mL]   (not used in calc.)
     Use Multiplier & Dilution Factor with ISTDs


     Signal 1: VWD1 A, Wavelength=476 nm
      Results obtained with enhanced integrator!

     RetTime   k'      Area       Height    Symm.  Width    Plates Resol Select
      [min]          mAU   *s    [mAU   ]         [min]           ution ivity
     -------|------|----------|----------|-----|-------|--------|-----|------
       4.462   -      14.66904    2.39120  0.88  0.0874   14452    -      -
       6.141   -      12.71297    1.62825  0.82  0.1177   15085  9.62   1.38
       7.474   -      14.60801    1.30481  0.65  0.1626   11708  5.59   1.22
      10.995   -      14.44422 9.29935e-1  0.69  0.2330   12341 10.46   1.47

     =================================================================
                           *** End of Report ***


Instrument 1 11/5/2009 10:11:34 AM soomer                                 Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\981104\SUDAN003.D                          Sample Name: 0

     =====================================================================
     Injection Date  : 11/5/2009 11:00:26 AM
     Sample Name     : 0                          Location : Vial 1
     Acq. Operator   : soomer
     Acq. Instrument : Instrument 1             Inj Volume : 10 µl
     Method          : C:\HPCHEM\1\METHODS\LIO-SU.M
     Last changed    : 11/5/2009 10:59:13 AM by soomer
                       (modified after loading)                           流速:1.3ml/mins
```

```
     =====================================================================
                     Area Percent Report with Performance
     =====================================================================

     Calib. Data Modified   :      Wednesday, May 16, 2007 3:41:33 PM
     Multiplier             :      1.0000
     Dilution               :      1.0000
     Sample Amount          :     10.00000  [ug/mL]   (not used in calc.)
     Use Multiplier & Dilution Factor with ISTDs

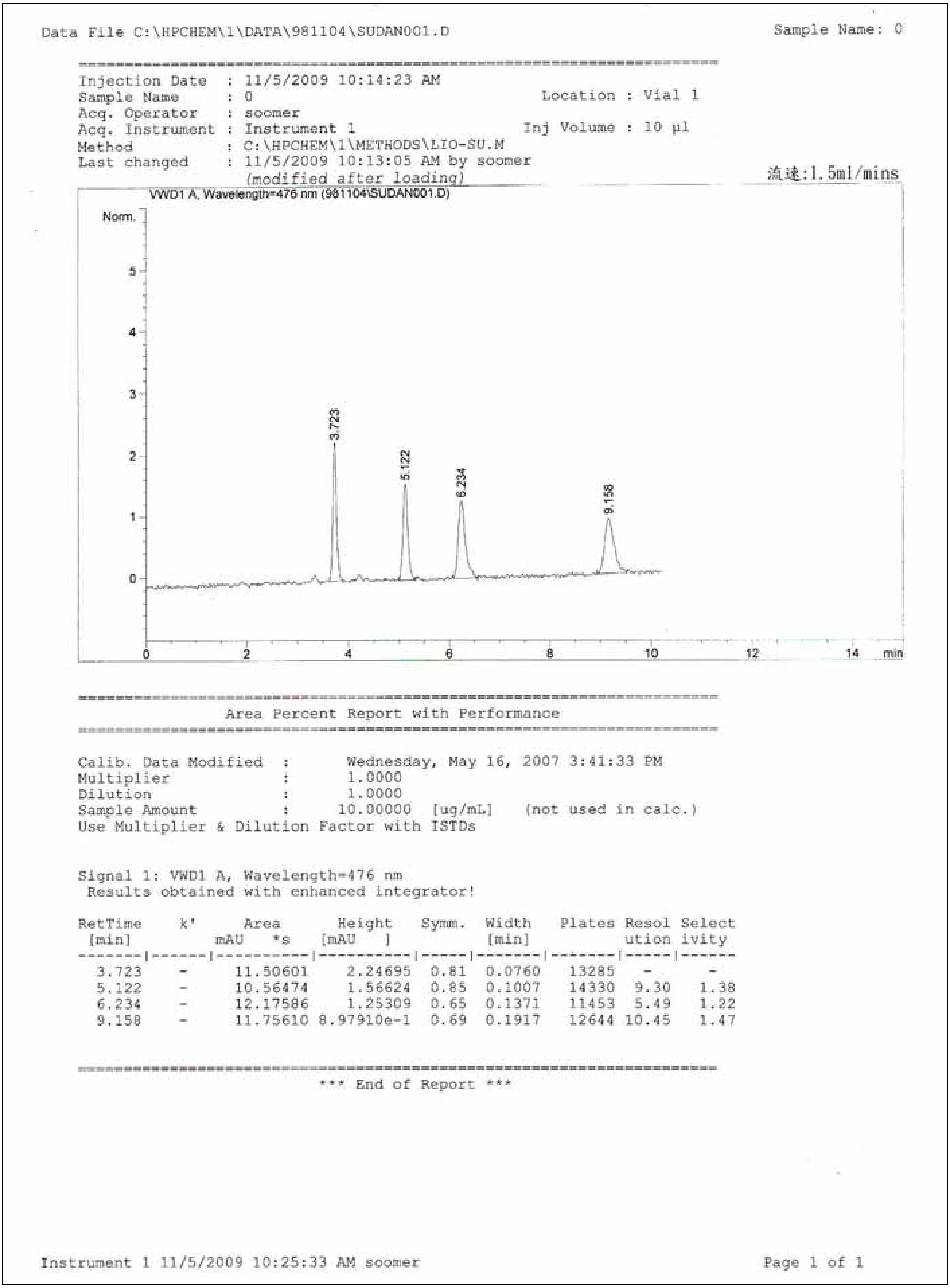

     Signal 1: VWD1 A, Wavelength=476 nm
      Results obtained with enhanced integrator!

     RetTime    k'     Area       Height    Symm.  Width    Plates Resol Select
      [min]          mAU   *s    [mAU   ]          [min]           ution ivity
     -------|------|----------|----------|-----|-------|-------|-----|------
       4.306   -      13.64280    2.30483  0.83  0.0861   13839   -      -
       5.925   -      12.31649    1.63947  0.82  0.1116   15610  9.62   1.38
       7.214   -      13.95513    1.25354  0.65  0.1638   10746  5.50   1.22
      10.609   -      13.93192 9.11433e-1  0.71  0.2184   13072 10.44   1.47

     =====================================================================
                              *** End of Report ***




Instrument 1 11/5/2009 11:13:49 AM soomer                          Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\981104\SUDAN001.D                            Sample Name: 0

    =====================================================================
    Injection Date  : 11/5/2009 10:14:23 AM
    Sample Name     : 0                           Location : Vial 1
    Acq. Operator   : soomer
    Acq. Instrument : Instrument 1              Inj Volume : 10 µl
    Method          : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/5/2009 10:13:05 AM by soomer
                      (modified after loading)
```

流速:1.5ml/mins

```
    =====================================================================
                     Area Percent Report with Performance
    =====================================================================

    Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Sample Amount         :     10.00000  [ug/mL]   (not used in calc.)
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

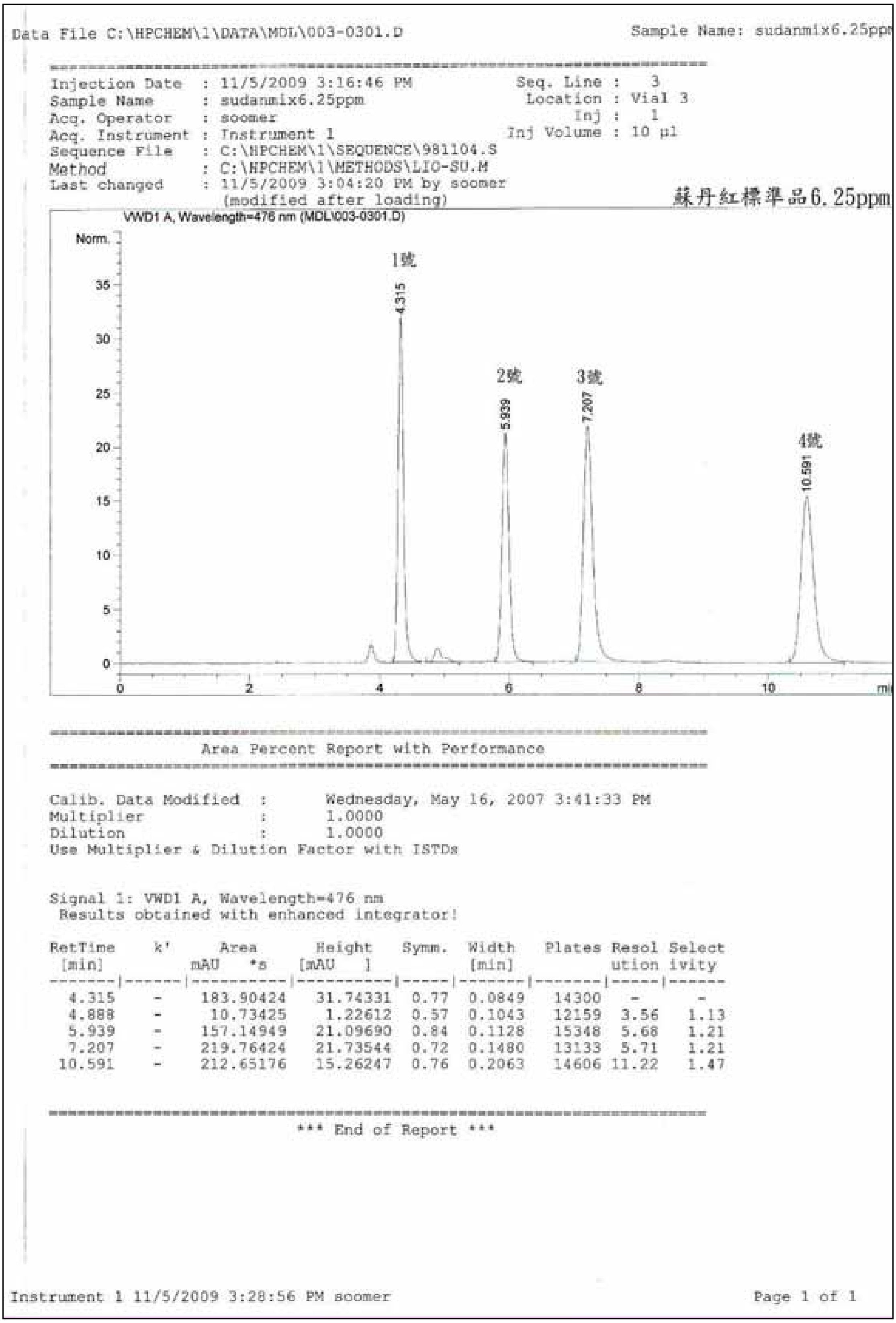
    RetTime    k'     Area       Height    Symm.  Width    Plates Resol Select
     [min]          mAU   *s    [mAU    ]         [min]           ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      3.723   -      11.50601     2.24695  0.81  0.0760   13285   -      -
      5.122   -      10.56474     1.56624  0.85  0.1007   14330  9.30   1.38
      6.234   -      12.17586     1.25309  0.65  0.1371   11453  5.49   1.22
      9.158   -      11.75610 8.97910e-1  0.69  0.1917   12644 10.45   1.47


    =====================================================================
                            *** End of Report ***




Instrument 1 11/5/2009 10:25:33 AM soomer                            Page 1 of 1
```

三、檢量線層析圖



```
Data File C:\HPCHEM\1\DATA\MDL\003-0301.D                        Sample Name: sudanmix6.25pp

   ==================================================================
   Injection Date   : 11/5/2009 3:16:46 PM            Seq. Line :   3
   Sample Name      : sudanmix6.25ppm                   Location : Vial 3
   Acq. Operator    : soomer                                 Inj :   1
   Acq. Instrument  : Instrument 1                    Inj Volume : 10 µl
   Sequence File    : C:\HPCHEM\1\SEQUENCE\981104.S
   Method           : C:\HPCHEM\1\METHODS\LIO-SU.M
   Last changed     : 11/5/2009 3:04:20 PM by soomer
                      (modified after loading)
```

蘇丹紅標準品6.25ppm

```
   ==================================================================
                  Area Percent Report with Performance
   ==================================================================

   Calib. Data Modified   :      Wednesday, May 16, 2007 3:41:33 PM
   Multiplier             :      1.0000
   Dilution               :      1.0000
   Use Multiplier & Dilution Factor with ISTDs

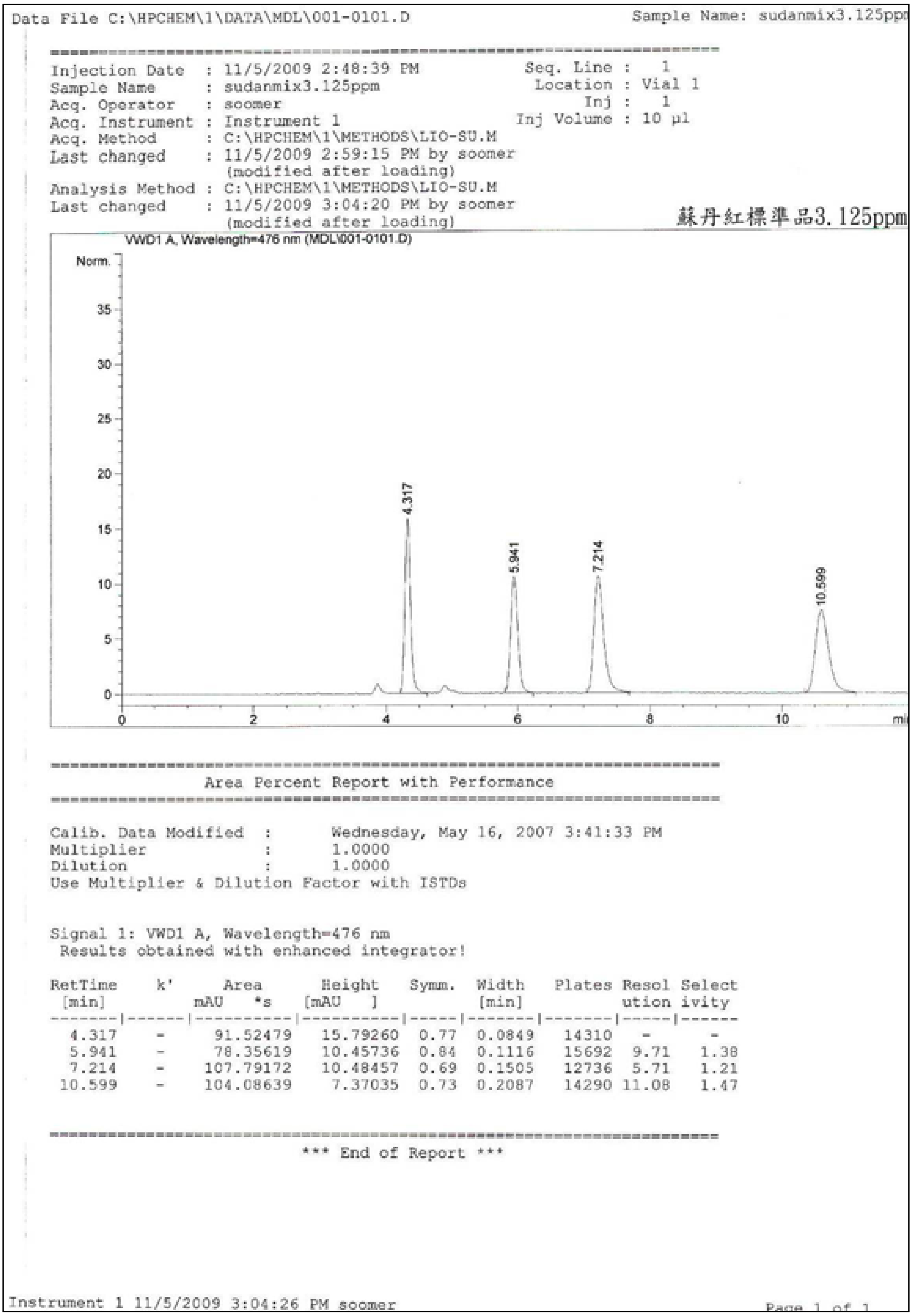

   Signal 1: VWD1 A, Wavelength=476 nm
    Results obtained with enhanced integrator!

   RetTime    k'     Area       Height    Symm.  Width   Plates Resol Select
    [min]          mAU   *s    [mAU  ]           [min]          ution ivity
   -------|------|-----------|----------|-----|-------|-------|-----|------
     4.315    -    183.90424   31.74331  0.77  0.0849   14300    -      -
     4.888    -     10.73425    1.22612  0.57  0.1043   12159  3.56   1.13
     5.939    -    157.14949   21.09690  0.84  0.1128   15348  5.68   1.21
     7.207    -    219.76424   21.73544  0.72  0.1480   13133  5.71   1.21
    10.591    -    212.65176   15.26247  0.76  0.2063   14606 11.22   1.47

   ==================================================================
                          *** End of Report ***



Instrument 1 11/5/2009 3:28:56 PM soomer                         Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\MDL\001-0101.D                    Sample Name: sudanmix3.125pp

    =====================================================================
    Injection Date   : 11/5/2009 2:48:39 PM              Seq. Line :   1
    Sample Name      : sudanmix3.125ppm                   Location : Vial 1
    Acq. Operator    : soomer                                  Inj :   1
    Acq. Instrument  : Instrument 1                     Inj Volume : 10 µl
    Acq. Method      : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed     : 11/5/2009 2:59:15 PM by soomer
                       (modified after loading)
    Analysis Method  : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed     : 11/5/2009 3:04:20 PM by soomer
                       (modified after loading)
```

蘇丹紅標準品3.125ppm

```
    =====================================================================
                    Area Percent Report with Performance
    =====================================================================

    Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs

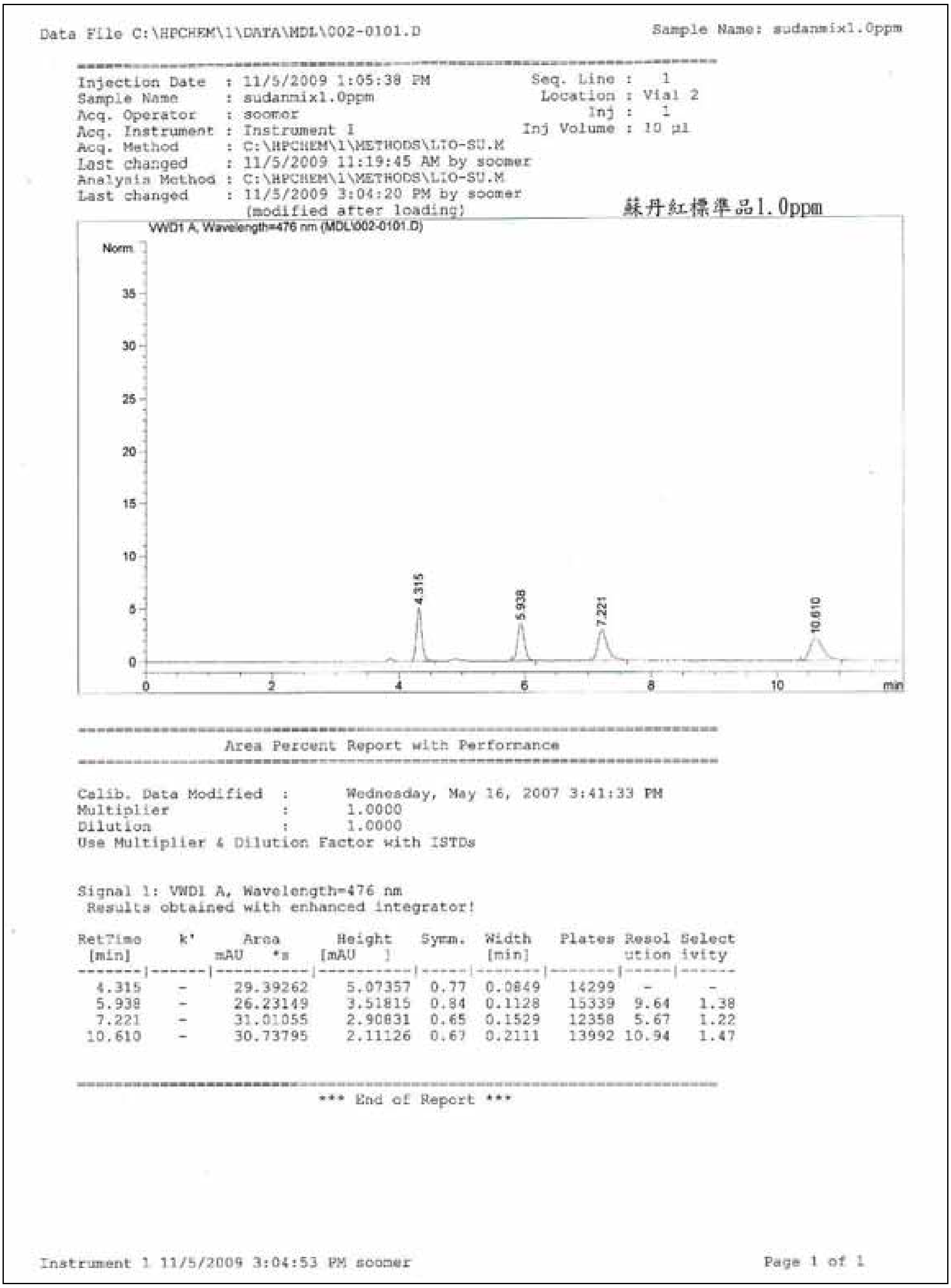

    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'     Area       Height    Symm.  Width   Plates Resol Select
     [min]          mAU   *s    [mAU   ]          [min]          ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      4.317    -     91.52479   15.79260  0.77  0.0849   14310   -      -
      5.941    -     78.35619   10.45736  0.84  0.1116   15692  9.71   1.38
      7.214    -    107.79172   10.48457  0.69  0.1505   12736  5.71   1.21
     10.599    -    104.08639    7.37035  0.73  0.2087   14290 11.08   1.47


    =====================================================================
                              *** End of Report ***




Instrument 1 11/5/2009 3:04:26 PM soomer                                   Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\MDL\002-0101.D                          Sample Name: sudanmix1.0ppm

    =====================================================================
    Injection Date  : 11/5/2009 1:05:38 PM              Seq. Line :   1
    Sample Name     : sudanmix1.0ppm                     Location : Vial 2
    Acq. Operator   : soomer                                  Inj :   1
    Acq. Instrument : Instrument I                     Inj Volume : 10 µl
    Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/5/2009 11:19:45 AM by soomer
    Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/5/2009 3:04:20 PM by soomer
                      (modified after loading)
```

蘇丹紅標準品1.0ppm

```
    =====================================================================
                   Area Percent Report with Performance
    =====================================================================

    Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs

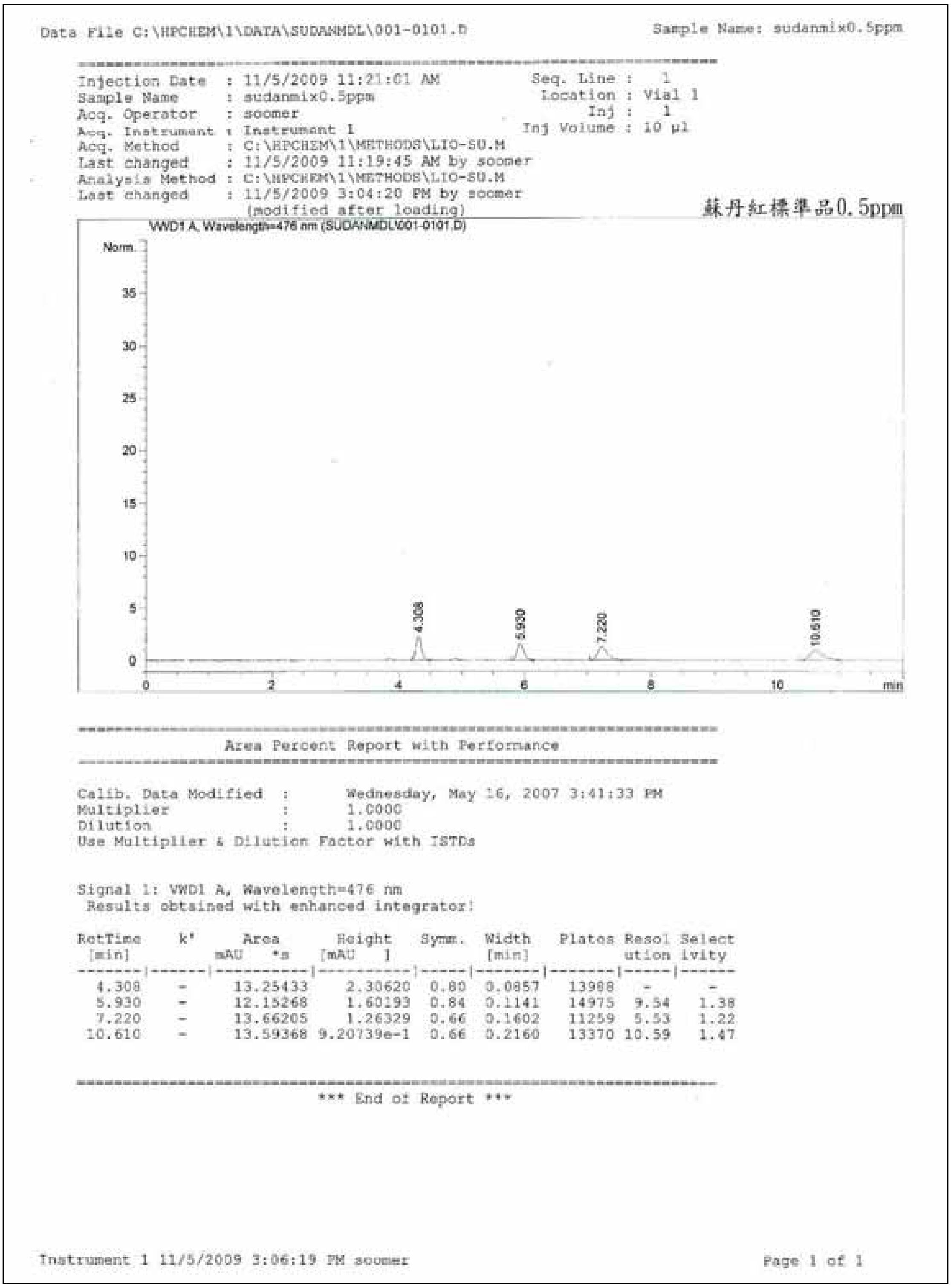

    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'      Area       Height    Symm.  Width    Plates Resol Select
     [min]           mAU   *s   [mAU    ]          [min]           ution ivity
    -------|------|----------|-----------|-----|--------|-------|-----|------
      4.315   -      29.39262    5.07357  0.77  0.0849   14299    -      -
      5.938   -      26.23149    3.51815  0.84  0.1128   15339  9.64   1.38
      7.221   -      31.01055    2.90831  0.65  0.1529   12358  5.67   1.22
     10.610   -      30.73795    2.11126  0.67  0.2111   13992 10.94   1.47


    =====================================================================
                            *** End of Report ***



Instrument 1 11/5/2009 3:04:53 PM soomer                                    Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\SUDANMDL\001-0101.D                    Sample Name: sudanmix0.5ppm

      ===================================================================
      Injection Date  : 11/5/2009 11:21:01 AM          Seq. Line :   1
      Sample Name     : sudanmix0.5ppm                  Location : Vial 1
      Acq. Operator   : soomer                               Inj :   1
      Acq. Instrument : Instrument 1                  Inj Volume : 10 µl
      Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
      Last changed    : 11/5/2009 11:19:45 AM by soomer
      Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
      Last changed    : 11/5/2009 3:04:20 PM by soomer
                        (modified after loading)
```

蘇丹紅標準品0.5ppm

```
      ===================================================================
                      Area Percent Report with Performance
      ===================================================================

      Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
      Multiplier            :      1.0000
      Dilution              :      1.0000
      Use Multiplier & Dilution Factor with ISTDs


      Signal 1: VWD1 A, Wavelength=476 nm
       Results obtained with enhanced integrator!

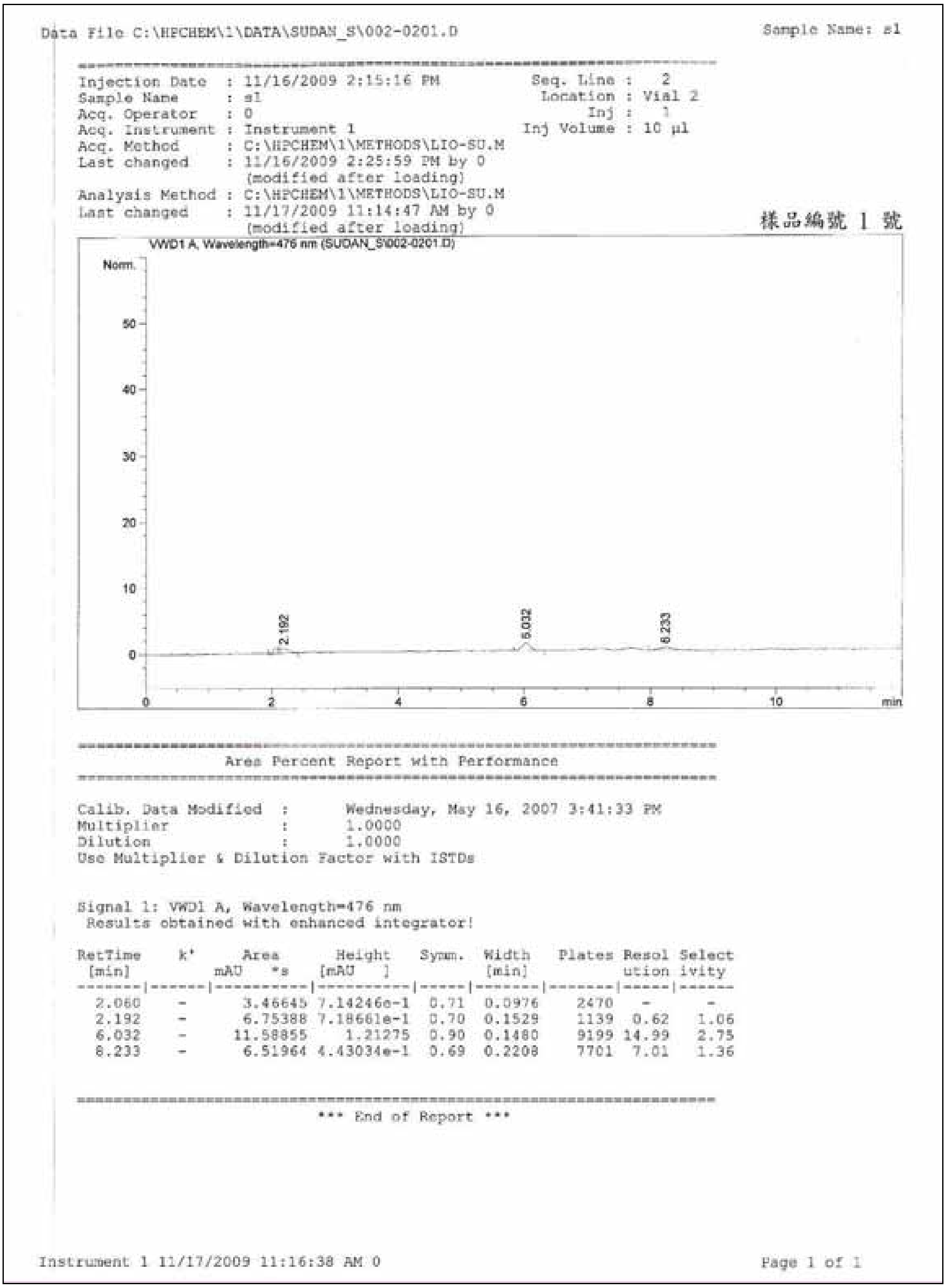
      RetTime    k'      Area      Height    Symm.  Width    Plates Resol Select
       [min]           mAU   *s   [mAU   ]          [min]           ution ivity
      -------|------|----------|----------|-----|-------|-------|-----|------
        4.308    -     13.25433    2.30620  0.80  0.0857   13988   -      -
        5.930    -     12.15268    1.60193  0.84  0.1141   14975  9.54   1.38
        7.220    -     13.66205    1.26329  0.66  0.1602   11259  5.53   1.22
       10.610    -     13.59368 9.20739e-1  0.66  0.2160   13370 10.59   1.47


      ===================================================================
                              *** End of Report ***



Instrument 1 11/5/2009 3:06:19 PM soomer                                    Page 1 of 1
```

四、檢體 1 號至 7 號層析圖



```
Data File C:\HPCHEM\1\DATA\SUDAN_S\002-0201.D                          Sample Name: s1

   =====================================================================
   Injection Date  : 11/16/2009 2:15:16 PM           Seq. Line :   2
   Sample Name     : s1                                Location : Vial 2
   Acq. Operator   : 0                                      Inj :   1
   Acq. Instrument : Instrument 1                    Inj Volume : 10 µl
   Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
   Last changed    : 11/16/2009 2:25:59 PM by 0
                     (modified after loading)
   Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
   Last changed    : 11/17/2009 11:14:47 AM by 0
                     (modified after loading)
```

樣品編號 1 號

```
   =====================================================================
                   Area Percent Report with Performance
   =====================================================================

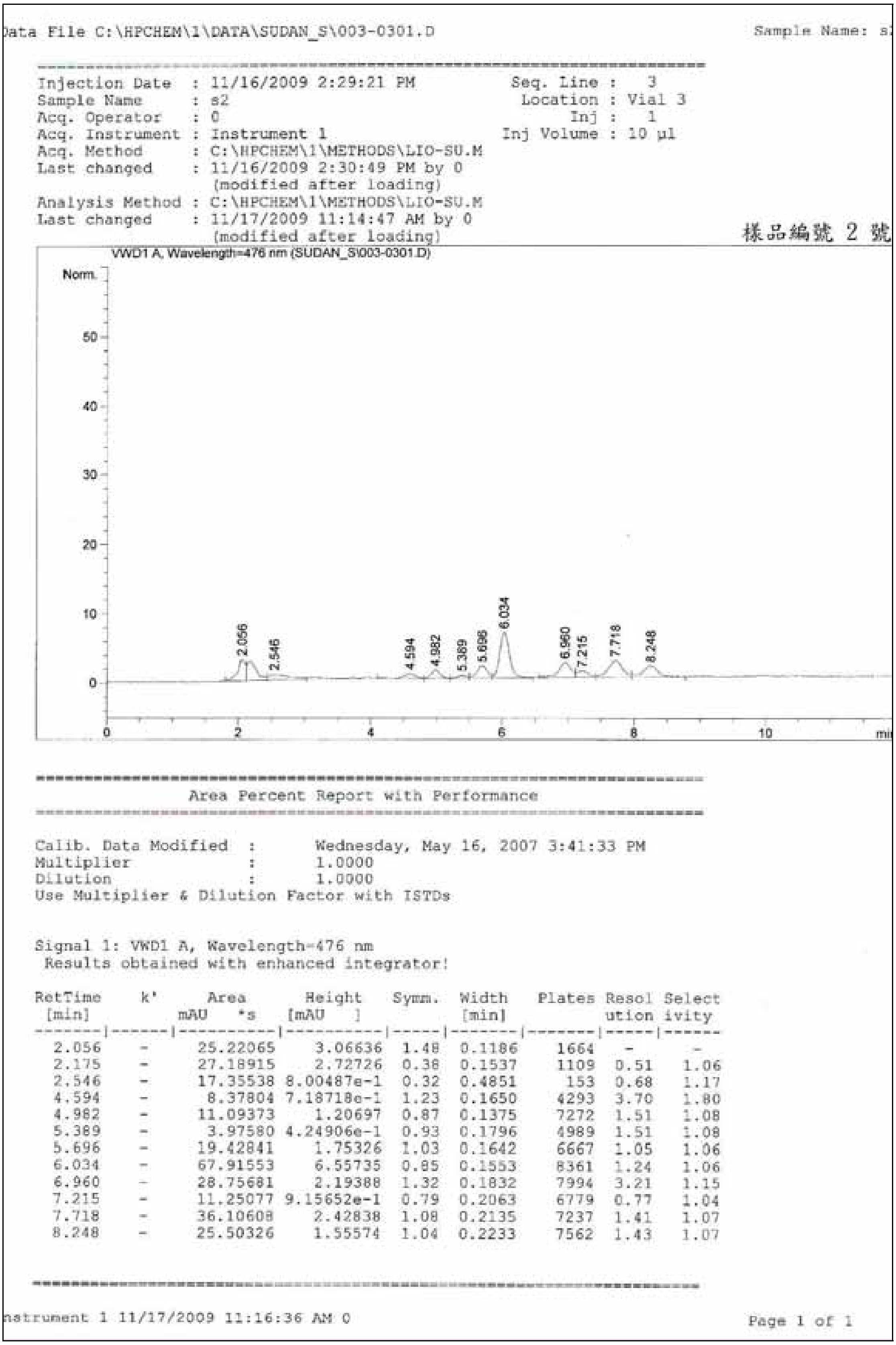
   Calib. Data Modified  :       Wednesday, May 16, 2007 3:41:33 PM
   Multiplier            :       1.0000
   Dilution              :       1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: VWD1 A, Wavelength=476 nm
    Results obtained with enhanced integrator!

   RetTime    k'     Area       Height     Symm.  Width    Plates Resol Select
    [min]          mAU   *s   [mAU   ]            [min]            ution ivity
   -------|------|----------|----------|-----|-------|-------|-----|------
     2.060   -      3.46645 7.14246e-1  0.71  0.0976    2470   -      -
     2.192   -      6.75388 7.18661e-1  0.70  0.1529    1139  0.62   1.06
     6.032   -     11.58855    1.21275  0.90  0.1480    9199 14.99   2.75
     8.233   -      6.51964 4.43034e-1  0.69  0.2208    7701  7.01   1.36


   =====================================================================
                            *** End of Report ***

Instrument 1 11/17/2009 11:16:38 AM 0                                  Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\SUDAN_S\003-0301.D                         Sample Name: s

   ========================================================================
   Injection Date  : 11/16/2009 2:29:21 PM          Seq. Line :   3
   Sample Name     : s2                              Location : Vial 3
   Acq. Operator   : 0                                    Inj :   1
   Acq. Instrument : Instrument 1                  Inj Volume : 10 µl
   Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
   Last changed    : 11/16/2009 2:30:49 PM by 0
                     (modified after loading)
   Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
   Last changed    : 11/17/2009 11:14:47 AM by 0
                     (modified after loading)
```

樣品編號 2 號

```
   ========================================================================
                   Area Percent Report with Performance
   ========================================================================

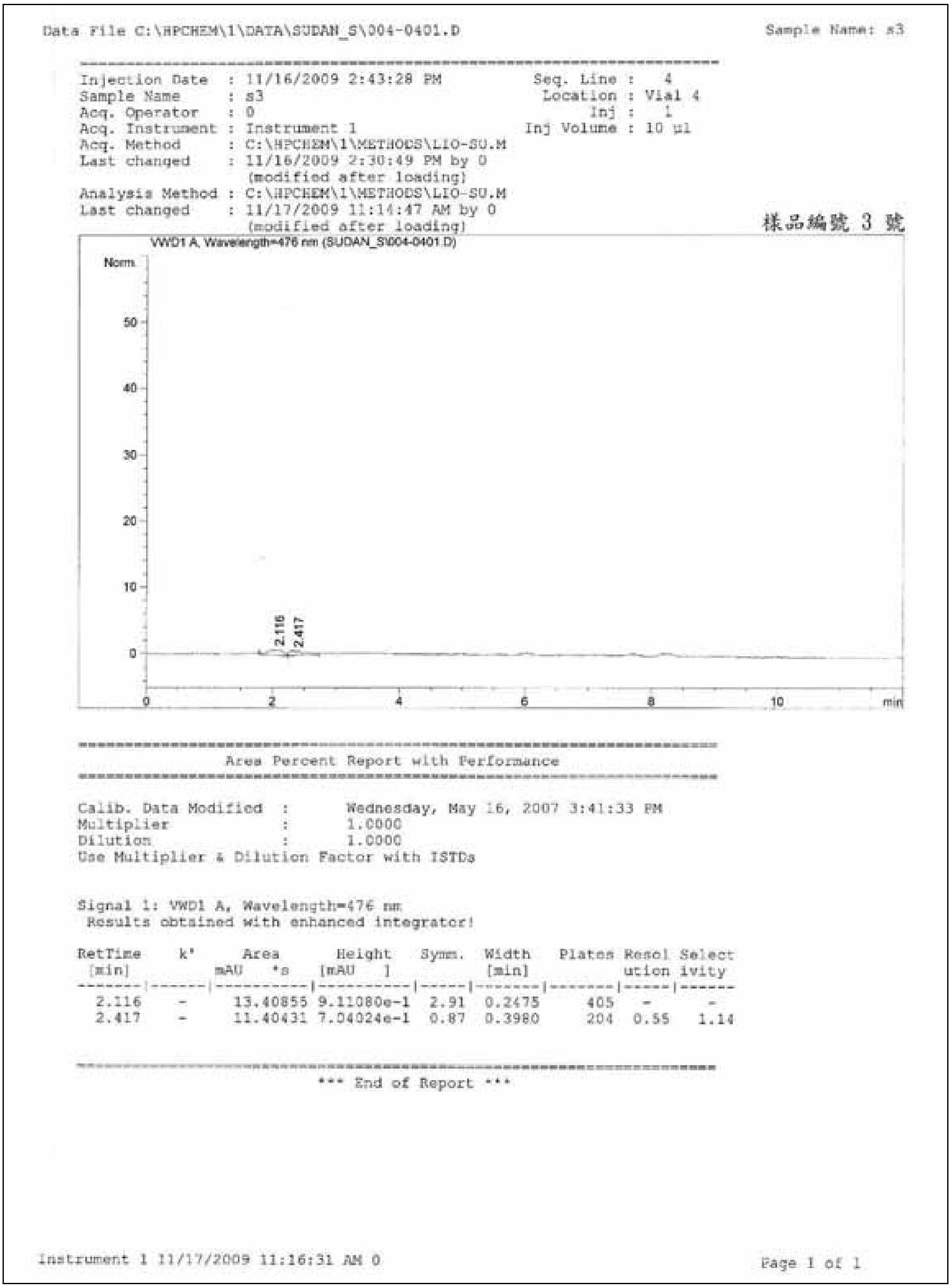
   Calib. Data Modified  :      Wednesday, May 16, 2007 3:41:33 PM
   Multiplier            :      1.0000
   Dilution              :      1.0000
   Use Multiplier & Dilution Factor with ISTDs


   Signal 1: VWD1 A, Wavelength=476 nm
    Results obtained with enhanced integrator!

   RetTime    k'     Area       Height     Symm.  Width    Plates Resol Select
    [min]          mAU   *s   [mAU    ]           [min]           ution ivity
   -------|------|----------|----------|-----|-------|-------|-----|------
     2.056    -     25.22065    3.06636  1.48  0.1186    1664   -      -
     2.175    -     27.18915    2.72726  0.38  0.1537    1109  0.51   1.06
     2.546    -     17.35538 8.00487e-1  0.32  0.4851     153  0.68   1.17
     4.594    -      8.37804 7.18718e-1  1.23  0.1650    4293  3.70   1.80
     4.982    -     11.09373    1.20697  0.87  0.1375    7272  1.51   1.08
     5.389    -      3.97580 4.24906e-1  0.93  0.1796    4989  1.51   1.08
     5.696    -     19.42841    1.75326  1.03  0.1642    6667  1.05   1.06
     6.034    -     67.91553    6.55735  0.85  0.1553    8361  1.24   1.06
     6.960    -     28.75681    2.19388  1.32  0.1832    7994  3.21   1.15
     7.215    -     11.25077 9.15652e-1  0.79  0.2063    6779  0.77   1.04
     7.718    -     36.10608    2.42838  1.08  0.2135    7237  1.41   1.07
     8.248    -     25.50326    1.55574  1.04  0.2233    7562  1.43   1.07


   ========================================================================

nstrument 1 11/17/2009 11:16:36 AM 0                                  Page 1 of 1
```



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    =====================================================================
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    Sample Name     : s3                                Location : Vial 4
    Acq. Operator   : 0                                      Inj :    1
    Acq. Instrument : Instrument 1                    Inj Volume : 10 µl
    Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
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                      (modified after loading)
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    Last changed    : 11/17/2009 11:14:47 AM by 0
                      (modified after loading)
```

樣品編號 3 號

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                     Area Percent Report with Performance
    =====================================================================

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    Dilution              :       1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'     Area      Height    Symm.  Width   Plates Resol Select
     [min]          mAU   *s   [mAU   ]          [min]           ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      2.116    -     13.40855 9.11080e-1  2.91  0.2475     405   -      -
      2.417    -     11.40431 7.04024e-1  0.87  0.3980     204  0.55   1.14

    =====================================================================
                              *** End of Report ***
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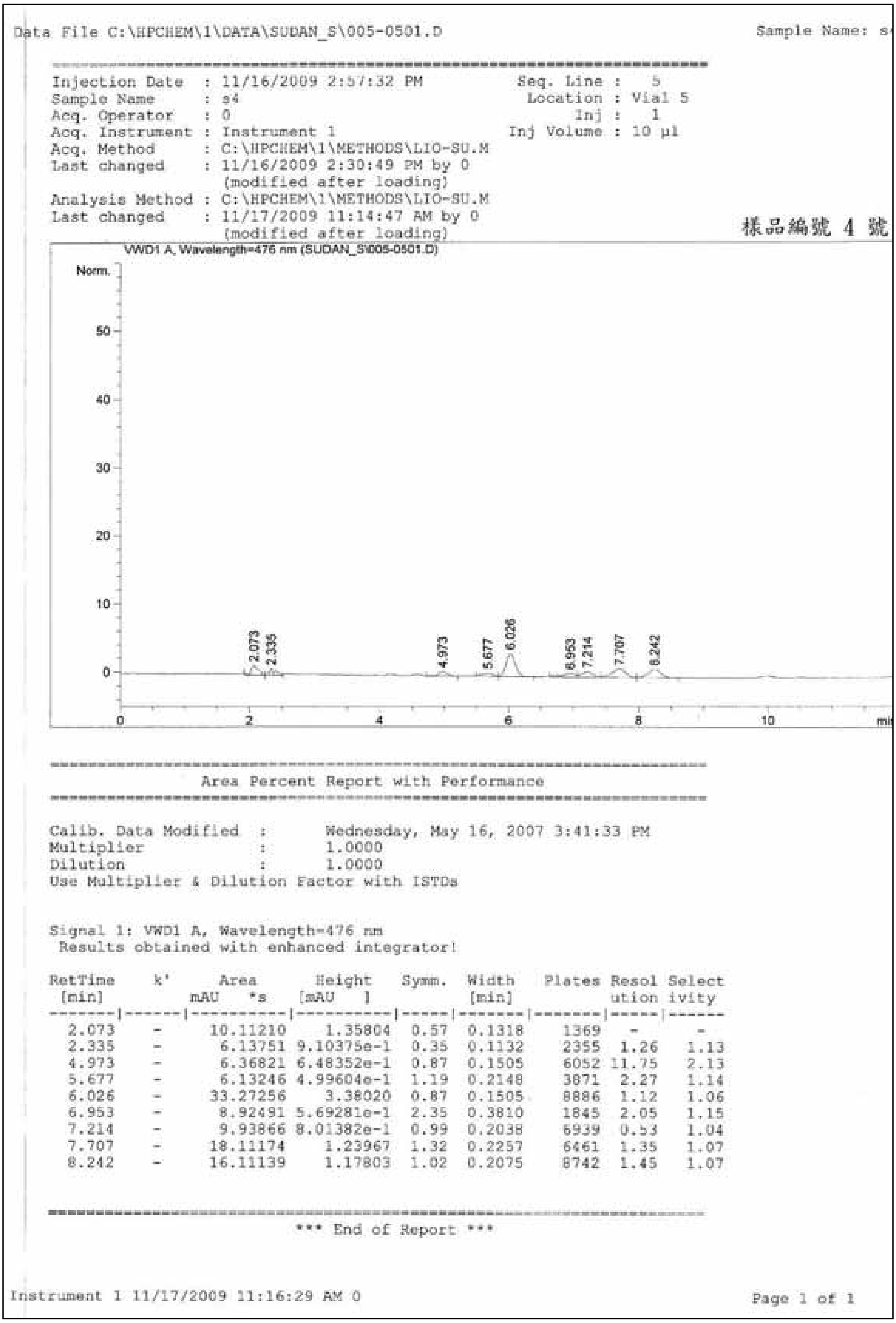
Instrument 1 11/17/2009 11:16:31 AM 0                                   Page 1 of 1
```



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Data File C:\HPCHEM\1\DATA\SUDAN_S\005-0501.D                                   Sample Name: s

    ======================================================================
    Injection Date   : 11/16/2009 2:57:32 PM          Seq. Line :    5
    Sample Name      : s4                              Location : Vial 5
    Acq. Operator    : 0                                    Inj :    1
    Acq. Instrument  : Instrument 1                  Inj Volume : 10 µl
    Acq. Method      : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed     : 11/16/2009 2:30:49 PM by 0
                       (modified after loading)
    Analysis Method  : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed     : 11/17/2009 11:14:47 AM by 0
                       (modified after loading)
```

樣品編號 4 號

```
    ======================================================================
                    Area Percent Report with Performance
    ======================================================================

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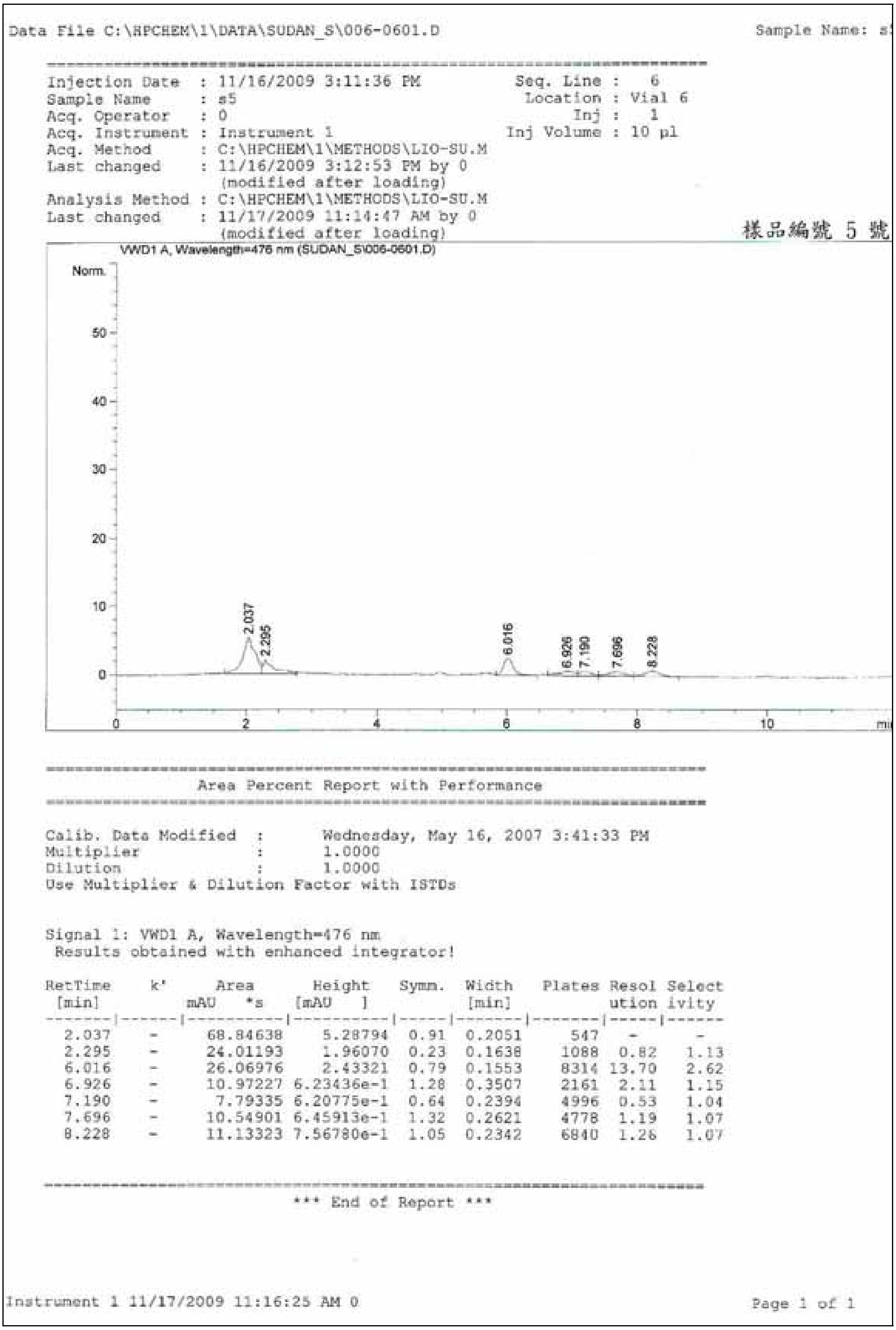
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'     Area      Height    Symm.  Width    Plates Resol Select
     [min]          mAU   *s   [mAU   ]          [min]           ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      2.073    -    10.11210    1.35804  0.57  0.1318    1369    -      -
      2.335    -     6.13751 9.10375e-1  0.35  0.1132    2355  1.26   1.13
      4.973    -     6.36821 6.48352e-1  0.87  0.1505    6052 11.75   2.13
      5.677    -     6.13246 4.99604e-1  1.19  0.2148    3871  2.27   1.14
      6.026    -    33.27256    3.38020  0.87  0.1505    8886  1.12   1.06
      6.953    -     8.92491 5.69281e-1  2.35  0.3810    1845  2.05   1.15
      7.214    -     9.93866 8.01382e-1  0.99  0.2038    6939  0.53   1.04
      7.707    -    18.11174    1.23967  1.32  0.2257    6461  1.35   1.07
      8.242    -    16.11139    1.17803  1.02  0.2075    8742  1.45   1.07


    ======================================================================
                            *** End of Report ***


Instrument 1 11/17/2009 11:16:29 AM 0                                        Page 1 of 1
```



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Data File C:\HPCHEM\1\DATA\SUDAN_S\006-0601.D                            Sample Name: s

    =====================================================================
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    Sample Name     : s5                                Location : Vial 6
    Acq. Operator   : 0                                      Inj :    1
    Acq. Instrument : Instrument 1                    Inj Volume : 10 µl
    Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/16/2009 3:12:53 PM by 0
                      (modified after loading)
    Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/17/2009 11:14:47 AM by 0
                      (modified after loading)
```

樣品編號 5 號

```
    =====================================================================
                     Area Percent Report with Performance
    =====================================================================

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    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs

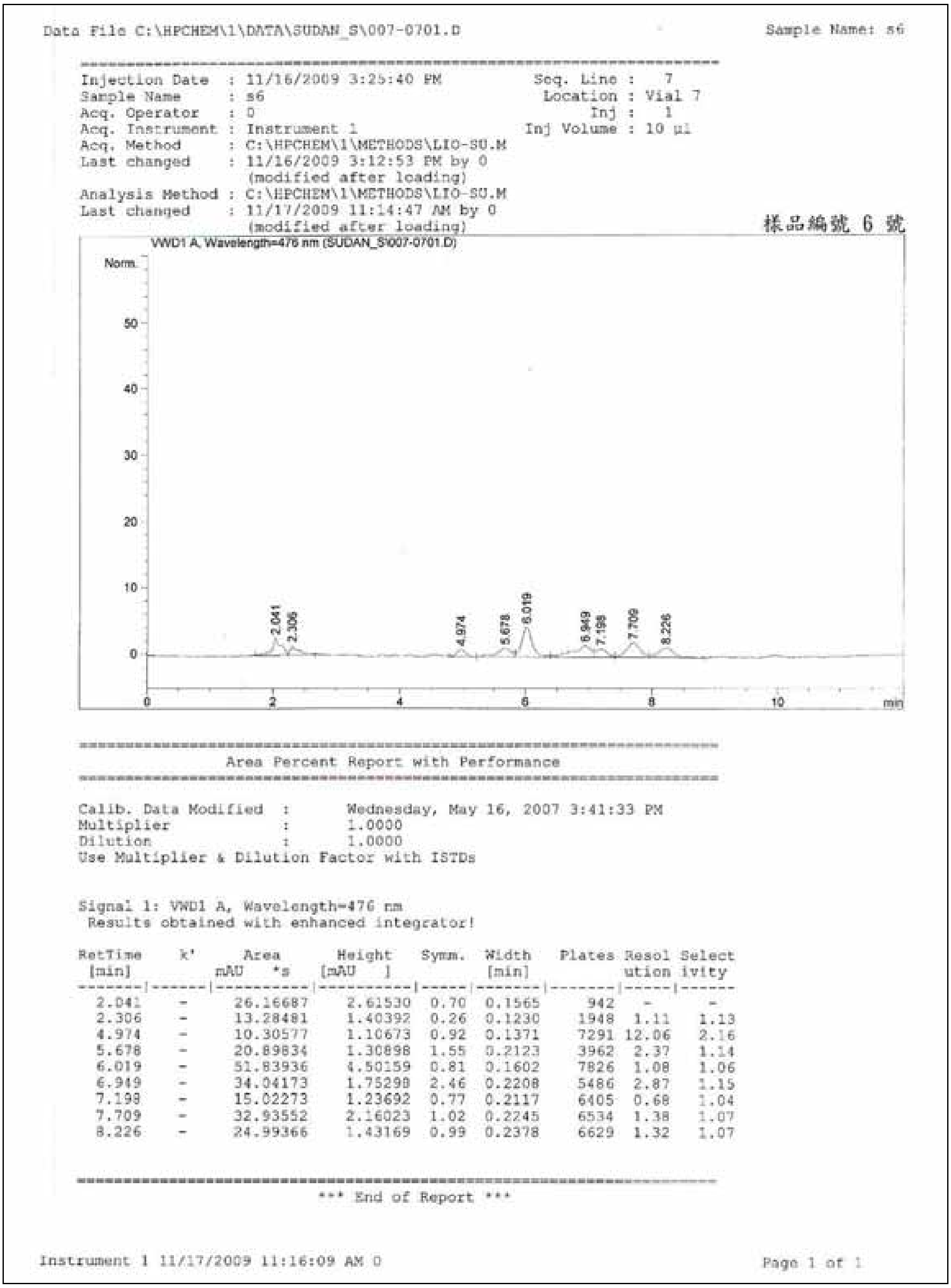

    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'     Area       Height     Symm.  Width    Plates Resol Select
     [min]          mAU   *s   [mAU    ]          [min]           ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      2.037    -     68.84638    5.28794  0.91  0.2051     547   -      -
      2.295    -     24.01193    1.96070  0.23  0.1638    1088  0.82   1.13
      6.016    -     26.06976    2.43321  0.79  0.1553    8314 13.70   2.62
      6.926    -     10.97227 6.23436e-1  1.28  0.3507    2161  2.11   1.15
      7.190    -      7.79335 6.20775e-1  0.64  0.2394    4996  0.53   1.04
      7.696    -     10.54901 6.45913e-1  1.32  0.2621    4778  1.19   1.07
      8.228    -     11.13323 7.56780e-1  1.05  0.2342    6840  1.26   1.07


    =====================================================================
                             *** End of Report ***



Instrument 1 11/17/2009 11:16:25 AM 0                                    Page 1 of 1
```



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Data File C:\HPCHEM\1\DATA\SUDAN_S\007-0701.D                              Sample Name: s6

     =====================================================================
     Injection Date  : 11/16/2009 3:25:40 PM          Seq. Line :    7
     Sample Name     : s6                              Location : Vial 7
     Acq. Operator   : 0                                    Inj :    1
     Acq. Instrument : Instrument 1                  Inj Volume : 10 µl
     Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
     Last changed    : 11/16/2009 3:12:53 PM by 0
                       (modified after loading)
     Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
     Last changed    : 11/17/2009 11:14:47 AM by 0
                       (modified after loading)
```

樣品編號 6 號

```
     =====================================================================
                     Area Percent Report with Performance
     =====================================================================

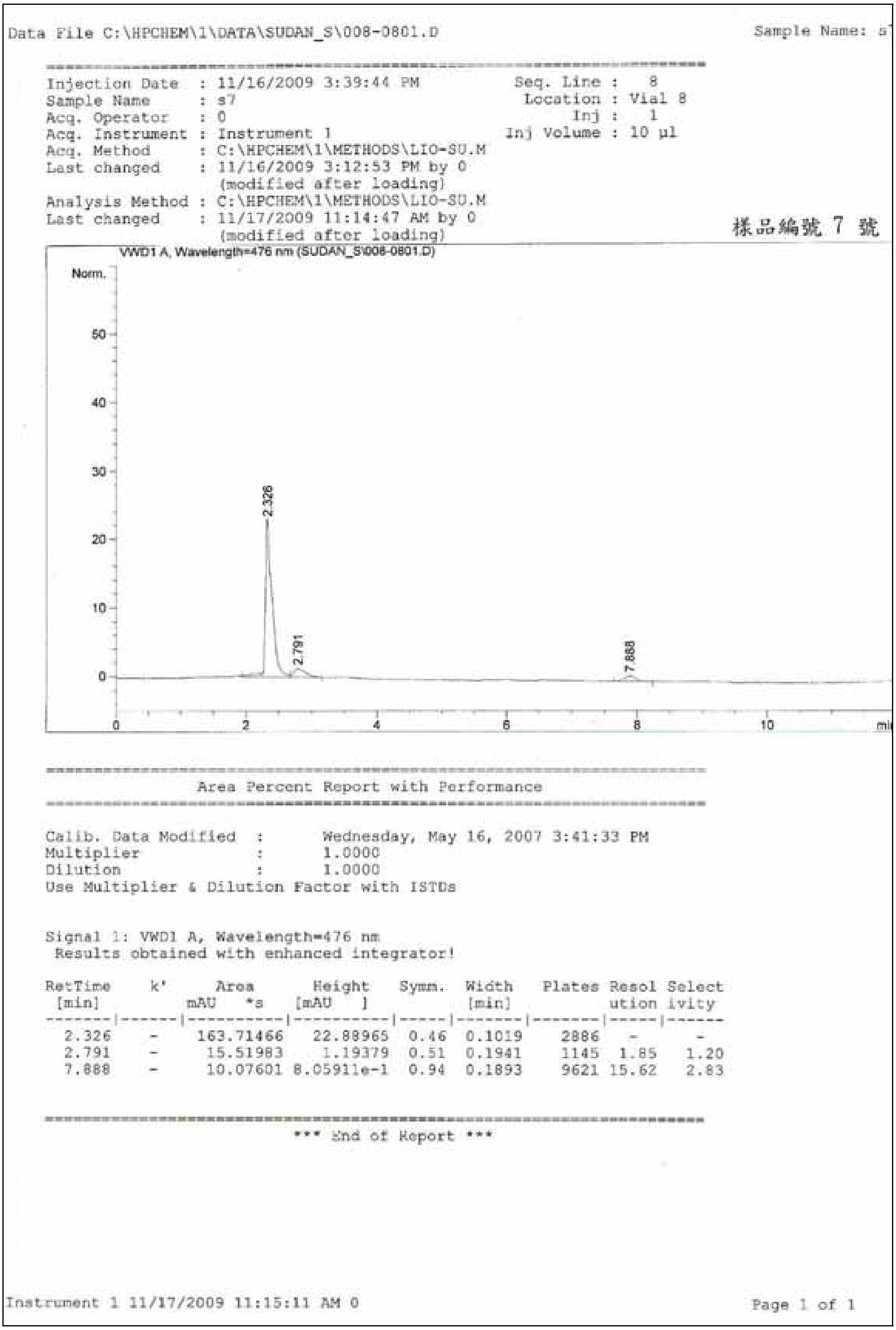
     Calib. Data Modified  :       Wednesday, May 16, 2007 3:41:33 PM
     Multiplier            :       1.0000
     Dilution              :       1.0000
     Use Multiplier & Dilution Factor with ISTDs


     Signal 1: VWD1 A, Wavelength=476 nm
      Results obtained with enhanced integrator!

     RetTime    k'     Area       Height    Symm.  Width    Plates Resol Select
      [min]          mAU   *s   [mAU   ]          [min]            ution ivity
     -------|------|----------|----------|-----|-------|-------|-----|------
       2.041    -     26.16687    2.61530  0.70  0.1565      942   -      -
       2.306    -     13.28481    1.40392  0.26  0.1230     1948  1.11   1.13
       4.974    -     10.30577    1.10673  0.92  0.1371     7291 12.06   2.16
       5.678    -     20.89834    1.30898  1.55  0.2123     3962  2.37   1.14
       6.019    -     51.83936    4.50159  0.81  0.1602     7826  1.08   1.06
       6.949    -     34.04173    1.75298  2.46  0.2208     5486  2.87   1.15
       7.198    -     15.02273    1.23692  0.77  0.2117     6405  0.68   1.04
       7.709    -     32.93552    2.16023  1.02  0.2245     6534  1.38   1.07
       8.226    -     24.99366    1.43169  0.99  0.2378     6629  1.32   1.07


     =====================================================================
                             *** End of Report ***


Instrument 1 11/17/2009 11:16:09 AM 0                                         Page 1 of 1
```



```
Data File C:\HPCHEM\1\DATA\SUDAN_S\008-0801.D                          Sample Name: s

    =====================================================================
    Injection Date  : 11/16/2009 3:39:44 PM            Seq. Line :   8
    Sample Name     : s7                                 Location : Vial 8
    Acq. Operator   : 0                                       Inj :   1
    Acq. Instrument : Instrument 1                     Inj Volume : 10 µl
    Acq. Method     : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/16/2009 3:12:53 PM by 0
                      (modified after loading)
    Analysis Method : C:\HPCHEM\1\METHODS\LIO-SU.M
    Last changed    : 11/17/2009 11:14:47 AM by 0
                      (modified after loading)
```

樣品編號 7 號

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    =====================================================================
                      Area Percent Report with Performance
    =====================================================================

    Calib. Data Modified   :     Wednesday, May 16, 2007 3:41:33 PM
    Multiplier             :     1.0000
    Dilution               :     1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: VWD1 A, Wavelength=476 nm
     Results obtained with enhanced integrator!

    RetTime    k'     Area      Height    Symm.  Width   Plates Resol Select
     [min]          mAU   *s   [mAU   ]          [min]          ution ivity
    -------|------|----------|----------|-----|-------|-------|-----|------
      2.326    -    163.71466   22.88965  0.46  0.1019    2886    -      -
      2.791    -     15.51983    1.19379  0.51  0.1941    1145  1.85   1.20
      7.888    -     10.07601 8.05911e-1  0.94  0.1893    9621 15.62   2.83


    =====================================================================
                              *** End of Report ***



Instrument 1 11/17/2009 11:15:11 AM 0                                   Page 1 of 1
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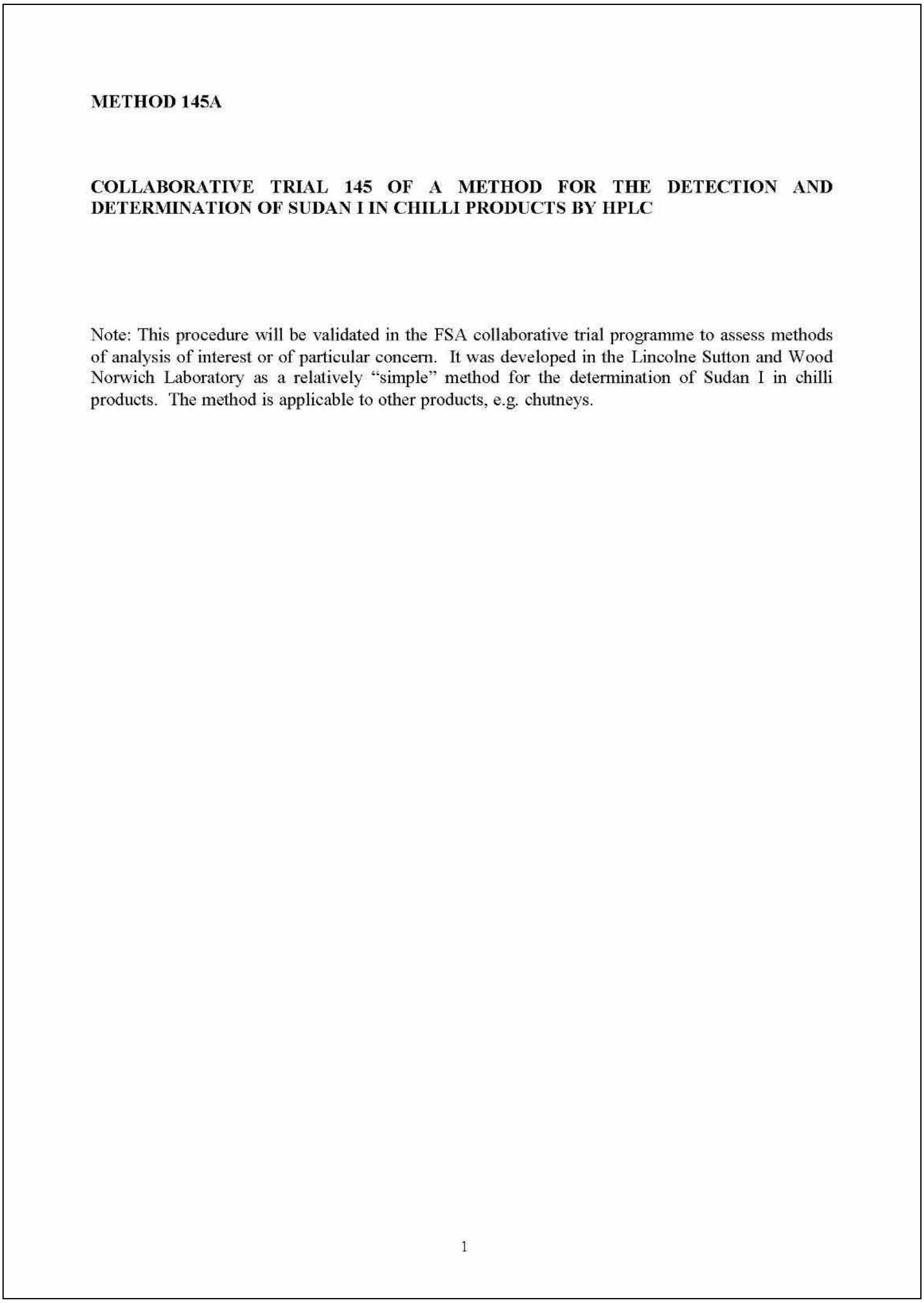
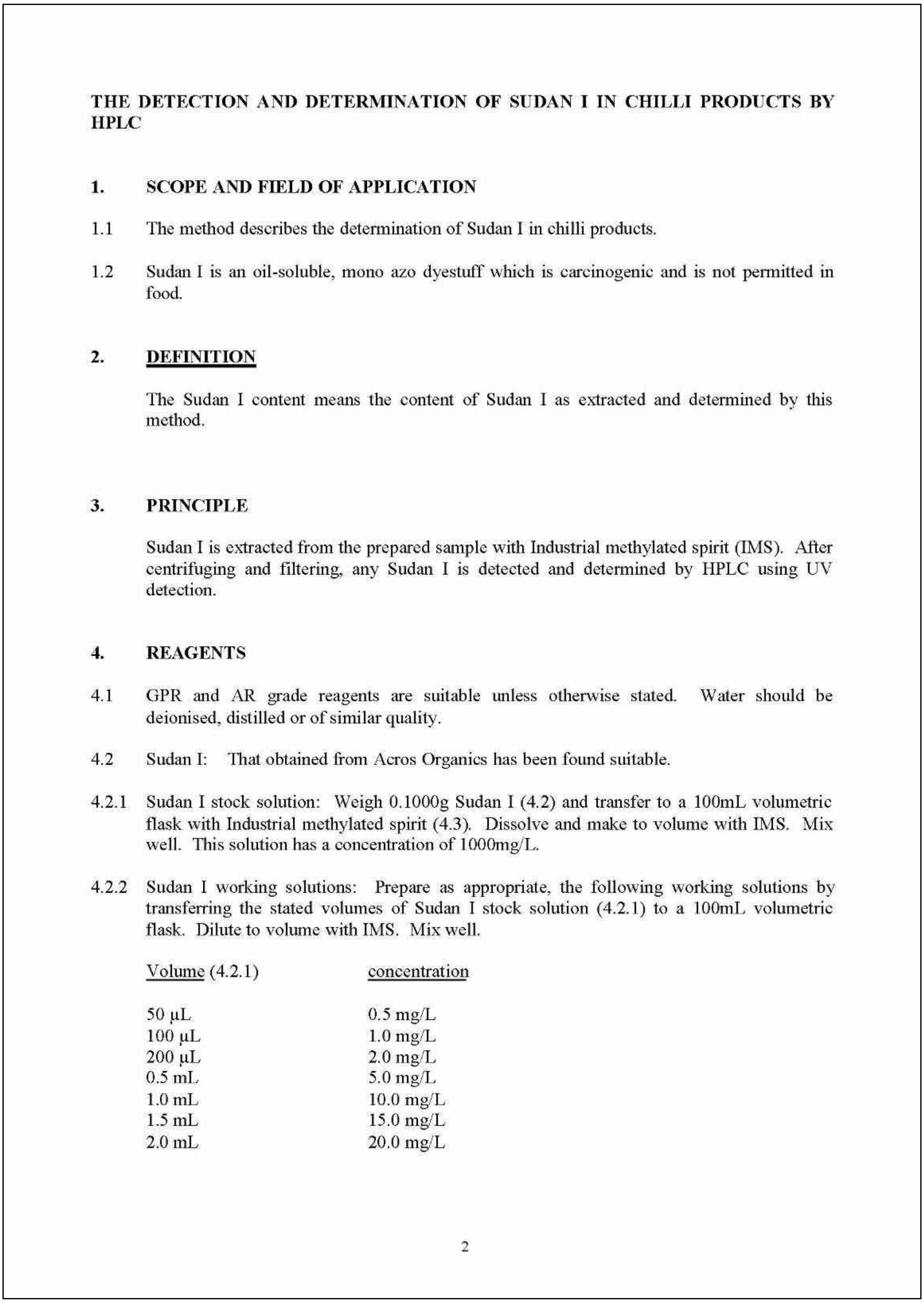
五、英國食品標準局(FOOD STANDARDS AGENCY)FSA145A 檢驗法原文



METHOD 145A

COLLABORATIVE TRIAL 145 OF A METHOD FOR THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC

Note: This procedure will be validated in the FSA collaborative trial programme to assess methods of analysis of interest or of particular concern. It was developed in the Lincolne Sutton and Wood Norwich Laboratory as a relatively "simple" method for the determination of Sudan I in chilli products. The method is applicable to other products, e.g. chutneys.



THE DETECTION AND DETERMINATION OF SUDAN I IN CHILLI PRODUCTS BY HPLC

1. SCOPE AND FIELD OF APPLICATION

1.1 The method describes the determination of Sudan I in chilli products.

1.2 Sudan I is an oil-soluble, mono azo dyestuff which is carcinogenic and is not permitted in food.

2. DEFINITION

The Sudan I content means the content of Sudan I as extracted and determined by this method.

3. PRINCIPLE

Sudan I is extracted from the prepared sample with Industrial methylated spirit (IMS). After centrifuging and filtering, any Sudan I is detected and determined by HPLC using UV detection.

4. REAGENTS

4.1 GPR and AR grade reagents are suitable unless otherwise stated. Water should be deionised, distilled or of similar quality.

4.2 Sudan I: That obtained from Acros Organics has been found suitable.

4.2.1 Sudan I stock solution: Weigh 0.1000g Sudan I (4.2) and transfer to a 100mL volumetric flask with Industrial methylated spirit (4.3). Dissolve and make to volume with IMS. Mix well. This solution has a concentration of 1000mg/L.

4.2.2 Sudan I working solutions: Prepare as appropriate, the following working solutions by transferring the stated volumes of Sudan I stock solution (4.2.1) to a 100mL volumetric flask. Dilute to volume with IMS. Mix well.

Volume (4.2.1)	concentration
50 µL	0.5 mg/L
100 µL	1.0 mg/L
200 µL	2.0 mg/L
0.5 mL	5.0 mg/L
1.0 mL	10.0 mg/L
1.5 mL	15.0 mg/L
2.0 mL	20.0 mg/L

Once prepared the solutions should be protected from the light as far as is practicable e.g., by wrapping in aluminium type foil.

4.3 Industrial Methylated Spirit 99 (IMS): That obtained from Tennants has been found suitable.

4.4 Chloroform: HPLC grade.

4.5 Acetonitrile: HPLC grade.

4.6 Ammonium acetate

4.7 Triethylamine

4.8 Methanol: HPLC grade.

4.9 0.05mol/L ammonium acetate in methanol: Dissolve 3.850g ammonium acetate (4.6) in methanol (4.8), dilute to volume with methanol in a 1 litre volumetric flask. Mix well.

4.10 Mobile phase for HPLC: Mix in the following proportions:

4 part of Acetonitrile (4.5), and
1 part of 0.05mol/L ammonium acetate in methanol (4.8)

Add 1mL triethylamine (4.7) per litre prepared.

5. APPARATUS

5.1 Normal laboratory glassware and apparatus.

5.2 Ultra Turrax mixer fitted with 18N shaft or suitable equivalent.

5.3 Centrifuge: the Jouan G412 operating at a setting of 3000 ± 100 has been found suitable.

5.4 Centrifuge tubes for (5.3) 50mL capacity.

5.5 Filter papers: GF/A 70mm diameter.

5.6 HPLC with UV detector (see Appendix 1).

5.7 Laboratory homogeniser.

5.8 Screw cap glass vials: 9mL capacity.

5.9 'Tall form' glass beakers 150mL capacity.

5.10 Timing device.

6. PROCEDURE

6.1 Preparation of test sample

6.1.1 Dry chilli products (e.g. chilli powder or crushed chilli) need only to be mixed thoroughly. No attempt should be made to macerate chilli powder in high speed blenders due to the potential formation of irritating aerosols.

6.1.2 Wet chilli-containing food products (e.g. relishes, chutneys) should be rendered as homogenous as possible in a suitable laboratory homogeniser (5.7)

6.1.3 Keep the prepared sample in an airtight, opaque plastic container and store it in such a way that deterioration and change in composition are avoided.

6.1.4 Analyse the prepared sample as soon as possible after homogenisation. Immediately prior to analysis the prepared sample should be mixed to ensure homogeneity.

6.2 Weigh 5 ± 0.1g prepared sample into a 150mL 'Tall form' glass beaker (5.9).

6.3 Add 50.0mL Industrial Methylated Spirit (IMS) (4.3) to the test portion.

6.4 Blend using the Ultra Turrax mixer (5.2) for 3 minutes (yellow/green setting).

6.5 Transfer to balanced centrifuge tubes (5.4) and centrifuge (5.3) for 10 minutes.

6.6 Filter the supernatant through a filter paper (5.5) into a glass vial (5.8).

6.7 Secure the screw top of the vial and protect from the light as far as practicable e.g. by wrapping in aluminium.

6.8 Set up the HPLC system (Appendix 1) and allow to stabilize for at least 1 hour.

Calibration

NB. Linearity has been previously established over the range of working standard solutions (4.2.2) and it is therefore not necessary to perform a full calibration on each occasion. A full linearity check, however, must be performed when new working standard solutions are prepared.

6.9 Sequentially inject an appropriate volume (15μL) of test portion extract (6.7) and the same appropriate volume of suitable working standard solutions (4.2.2). Ensure that duplicate injections of the extract are bracketed by standard injections.

6.10 Identify the compound of interest in the extract chromatogram by virtue of its retention time with reference to the working standard solution chromatogram (see Appendix 2 for a typical chromatogram).

6.11 Record the areas of any identified peaks from the test portion extract chromatograms (6.9).

6.12 Record the areas of the peak of interest from the working standard solution chromatograms (6.9).

6.13 If the concentration of Sudan I in the test portion extract exceeds the highest working standard solution it is necessary to dilute a suitable aliquot to a known volume with IMS.

7. CALCULATION

For 5g □ 50

Concentration (mg/kg):

$$\frac{A}{B} \times Y \times 10 \times \frac{100}{WRm} \times D$$

Where

A = mean area of any identified peak in the test portion extract.

B = mean area of peak of interest in the working standard solution.

Y = concentration (mg/L) of the suitable working standard solution

WRm = working recovery mean (9.1.2)

D = the dilution factor (if any) (6.13)

8. EXPRESSION OF RESULTS

Record the result to the nearest 1 mg/kg.

9. ANALYTICAL QUALITY ASSURANCE

9.1 Performance Characteristics (Typical)

9.1.1 Limit of detection: to be assessed in the light of the collaborative trial.

9.1.2 Bias

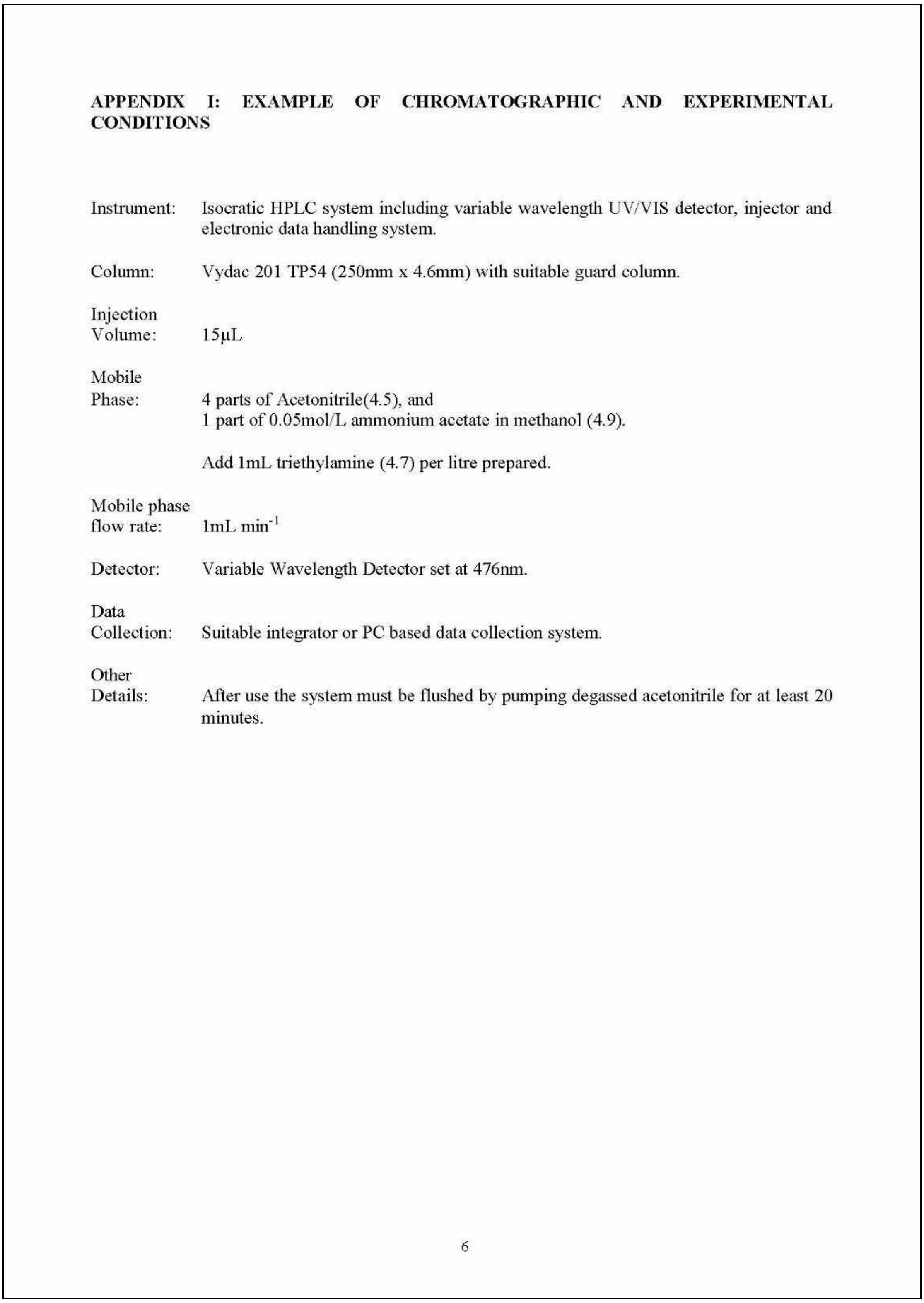
WRm(n=12): 105

WRs: 8.3

9.1.3 Precision

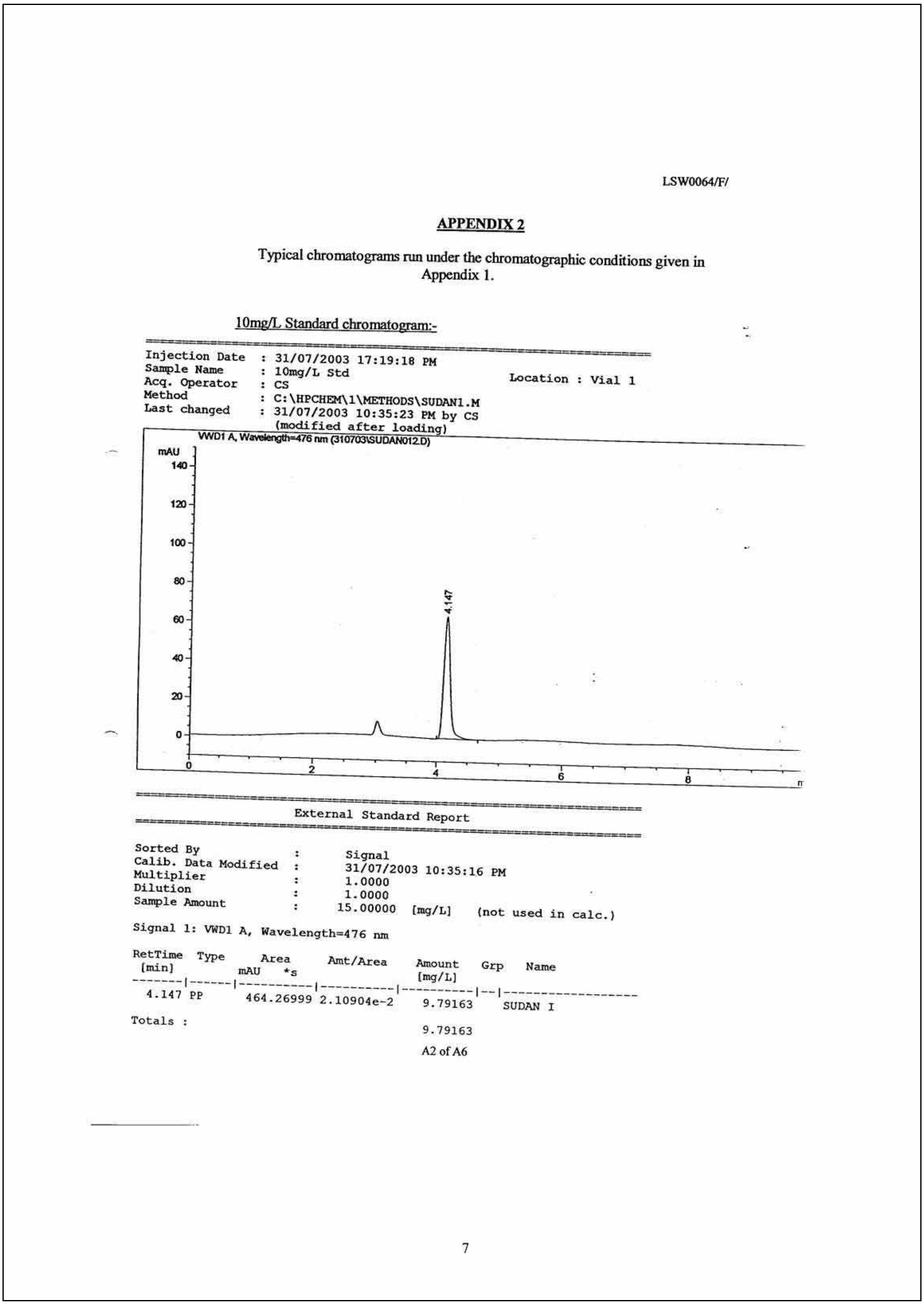
Wp: to be determined

Wp(relative): to be determined



APPENDIX I: EXAMPLE OF CHROMATOGRAPHIC AND EXPERIMENTAL CONDITIONS

Instrument:	Isocratic HPLC system including variable wavelength UV/VIS detector, injector and electronic data handling system.
Column:	Vydac 201 TP54 (250mm x 4.6mm) with suitable guard column.
Injection Volume:	15µL
Mobile Phase:	4 parts of Acetonitrile(4.5), and 1 part of 0.05mol/L ammonium acetate in methanol (4.9). Add 1mL triethylamine (4.7) per litre prepared.
Mobile phase flow rate:	1mL min^{-1}
Detector:	Variable Wavelength Detector set at 476nm.
Data Collection:	Suitable integrator or PC based data collection system.
Other Details:	After use the system must be flushed by pumping degassed acetonitrile for at least 20 minutes.



APPENDIX 2

Typical chromatograms run under the chromatographic conditions given in Appendix 1.

10mg/L Standard chromatogram:-

```
Injection Date   : 31/07/2003 17:19:18 PM
Sample Name      : 10mg/L Std
Acq. Operator    : CS                          Location : Vial 1
Method           : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed     : 31/07/2003 10:35:23 PM by CS
                   (modified after loading)
```

```
====================================================================
                    External Standard Report
====================================================================

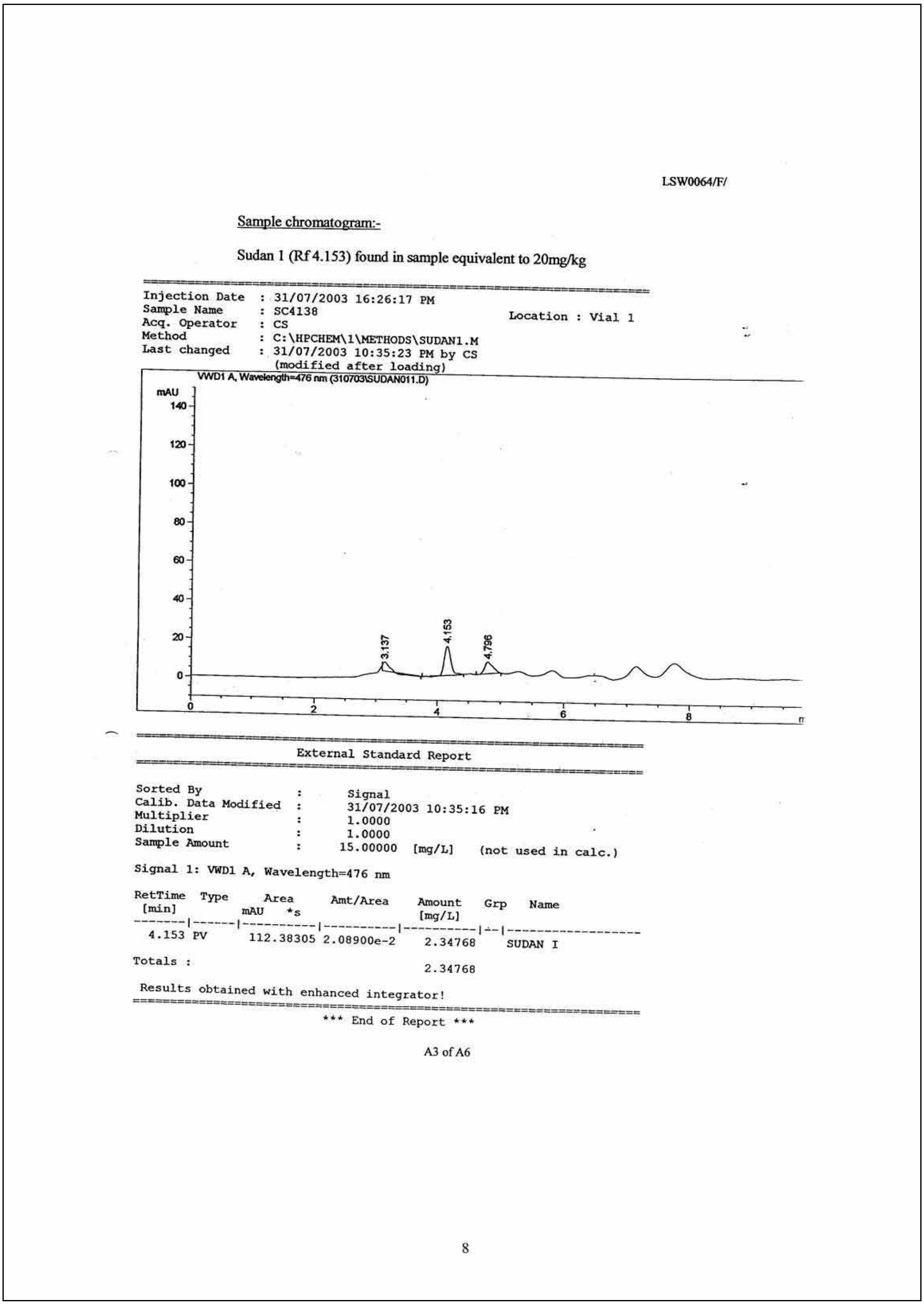
Sorted By              :       Signal
Calib. Data Modified   :       31/07/2003 10:35:16 PM
Multiplier             :       1.0000
Dilution               :       1.0000
Sample Amount          :      15.00000  [mg/L]   (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          mAU   *s                 [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.147 PP     464.26999 2.10904e-2   9.79163     SUDAN I

Totals :                              9.79163
```

A2 of A6



LSW0064/F/

Sample chromatogram:-

Sudan 1 (Rf 4.153) found in sample equivalent to 20mg/kg

```
===================================================================
Injection Date  : 31/07/2003 16:26:17 PM
Sample Name     : SC4138                          Location : Vial 1
Acq. Operator   : CS
Method          : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed    : 31/07/2003 10:35:23 PM by CS
                  (modified after loading)
```

```
=====================================================================
                    External Standard Report
=====================================================================

Sorted By               :      Signal
Calib. Data Modified    :      31/07/2003 10:35:16 PM
Multiplier              :      1.0000
Dilution                :      1.0000
Sample Amount           :     15.00000  [mg/L]    (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

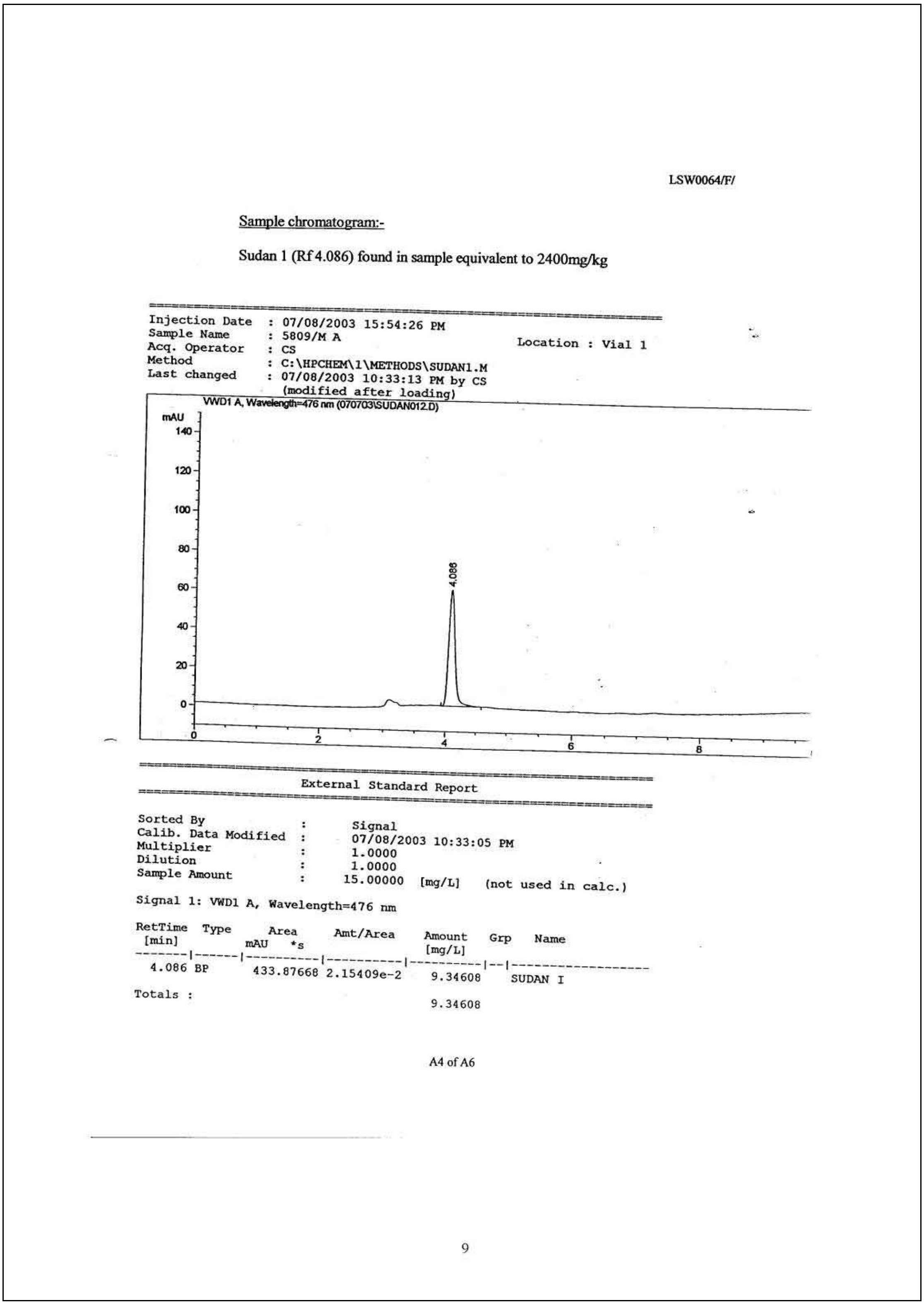
RetTime  Type     Area     Amt/Area     Amount    Grp   Name
 [min]          mAU   *s               [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.153 PV     112.38305 2.08900e-2    2.34768     SUDAN I

Totals :                               2.34768

Results obtained with enhanced integrator!
=====================================================================
                      *** End of Report ***
```

A3 of A6

8



LSW0064/F/

Sample chromatogram:-

Sudan 1 (Rf 4.086) found in sample equivalent to 2400mg/kg

```
=====================================================================
Injection Date  : 07/08/2003 15:54:26 PM
Sample Name     : 5809/M A                          Location : Vial 1
Acq. Operator   : CS
Method          : C:\HPCHEM\1\METHODS\SUDAN1.M
Last changed    : 07/08/2003 10:33:13 PM by CS
                  (modified after loading)
```

```
=====================================================================
                      External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      07/08/2003 10:33:05 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount         :     15.00000  [mg/L]   (not used in calc.)

Signal 1: VWD1 A, Wavelength=476 nm

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          mAU   *s                 [mg/L]
-------|------|----------|----------|----------|--|------------------
  4.086 BP     433.87668 2.15409e-2    9.34608     SUDAN I

Totals :                               9.34608
```

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六、標準品 Certificate of Analysis 資料

SIGMA-ALDRICH

Fluka Analytical

Industriestrasse 25, CH-9471 Buchs (SG), Switzerland
Tel: +41 81 755 2511 Fax: +41 81 756 5449

Certificate of Analysis

Product Name:	SUDAN I ANALYTICAL STANDARD
Product Number:	51383
Product Brand:	Fluka
Molecular Formula:	$C_{16}H_{12}N_2O$
Molecular Mass:	248.28
CAS Number:	842-07-9

TEST	**SPECIFICATION**	**LOT 1304322 RESULTS**
APPEARANCE (COLOR)	RED/BROWN-RED TO DEEP RED/BROWN--RED	DEEP RED
APPEARANCE (FORM)	POWDER TO FINE CRYSTALS	POWDER
PURITY (HPLC AREA %)	≥ 96.0 %	98.3 %
PROTON NMR SPECTRUM	CORRESPONDS TO STANDARD SPECTRUM	CONFORMS

QC RELEASE DATE	31/AUG/06
REASSAYED ON	03/JUL/09
RECOMMENDED RETEST DATE	JUN/12

Edeltraud Schwärzler, Manager
Quality Control
Buchs, Switzerland

Sigma-Aldrich Certificate of Analysis - Product 51383 Lot 1304322 Page 1 of 1

Certificate Of Analysis

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SIGMA-ALDRICH

Certificate of Analysis

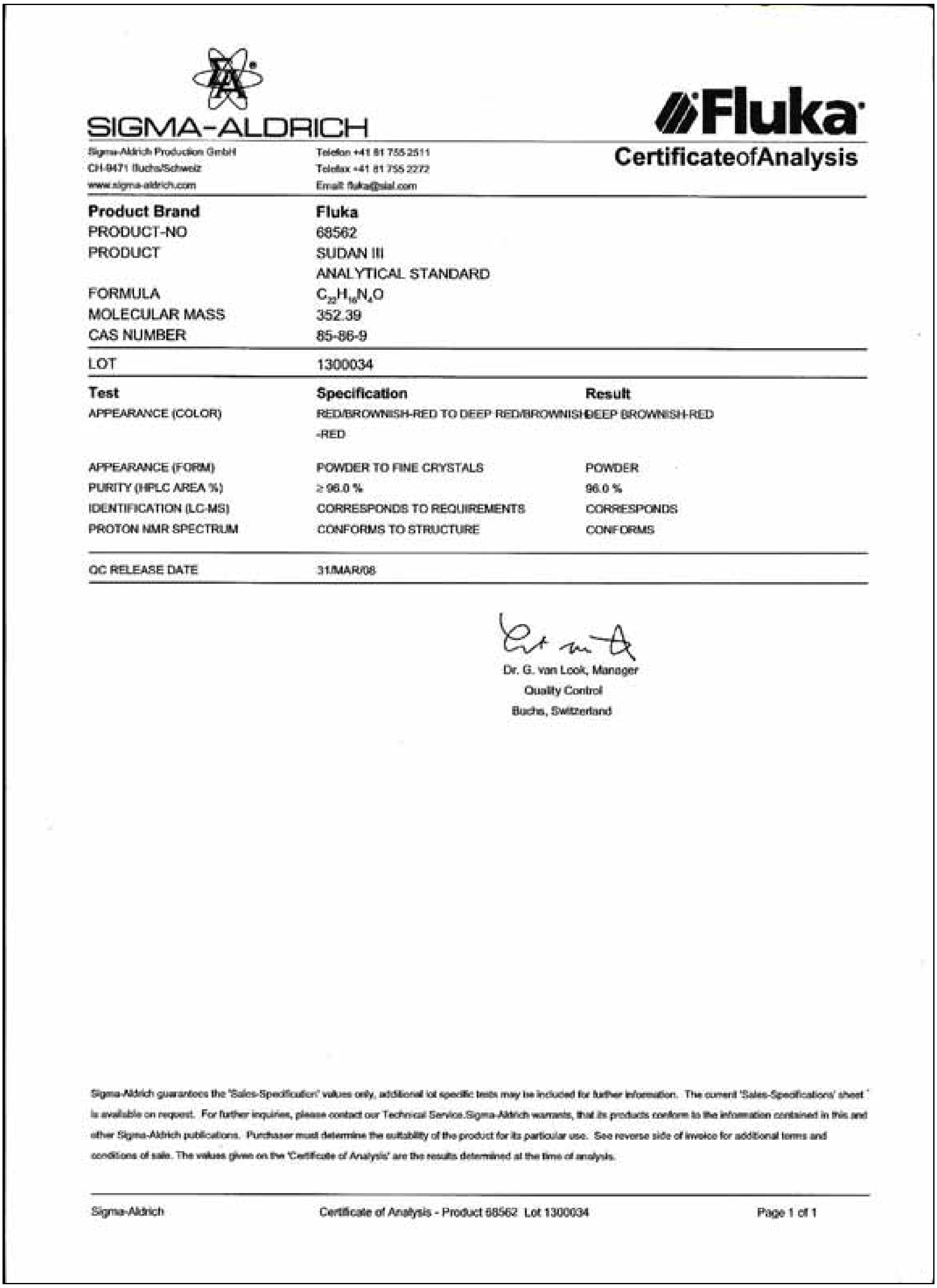
Product Name	Sudan II, analytical standard, ≥96.0% (HPLC)
Product Number	07937
Product Brand	Fluka
CAS Number	3118-97-6
Molecular Formula	$C_{18}H_{16}N_2O$
Molecular Weight	276.33

TEST	**LOT 1300029 RESULTS**
APPEARANCE (COLOR)	RED
APPEARANCE (FORM)	NEEDLES
PURITY (HPLC AREA %)	99.3 % rel
IDENTIFICATION (LC-MS)	CORRESPONDS
NMR SPECTRUM 1H	corresponds
DATE OF QC-RELEASE	10/JAN/07

Sigma-Aldrich guarantees the 'Sales-Specification' values only, non-specified tests may be included as additional information. The current 'Sales-Specifications' sheet is available on request. For further inquiries, please contact our Technical Service. Sigma-Aldrich warrants, that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice for additional terms and conditions of sale. The values given on the 'Certificate of Analysis' are the results determined at the time of analysis.

Dr. Gert van Look, Manager
Quality Control
Buchs Switzerland

http://www5.sigmaaldrich.com/catalog/search/CertOfAnalysisPage/07937?LotNo=1300029&PrtPrv=1 2009/8/19



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Email fluka@sial.com

Fluka

Certificate of Analysis

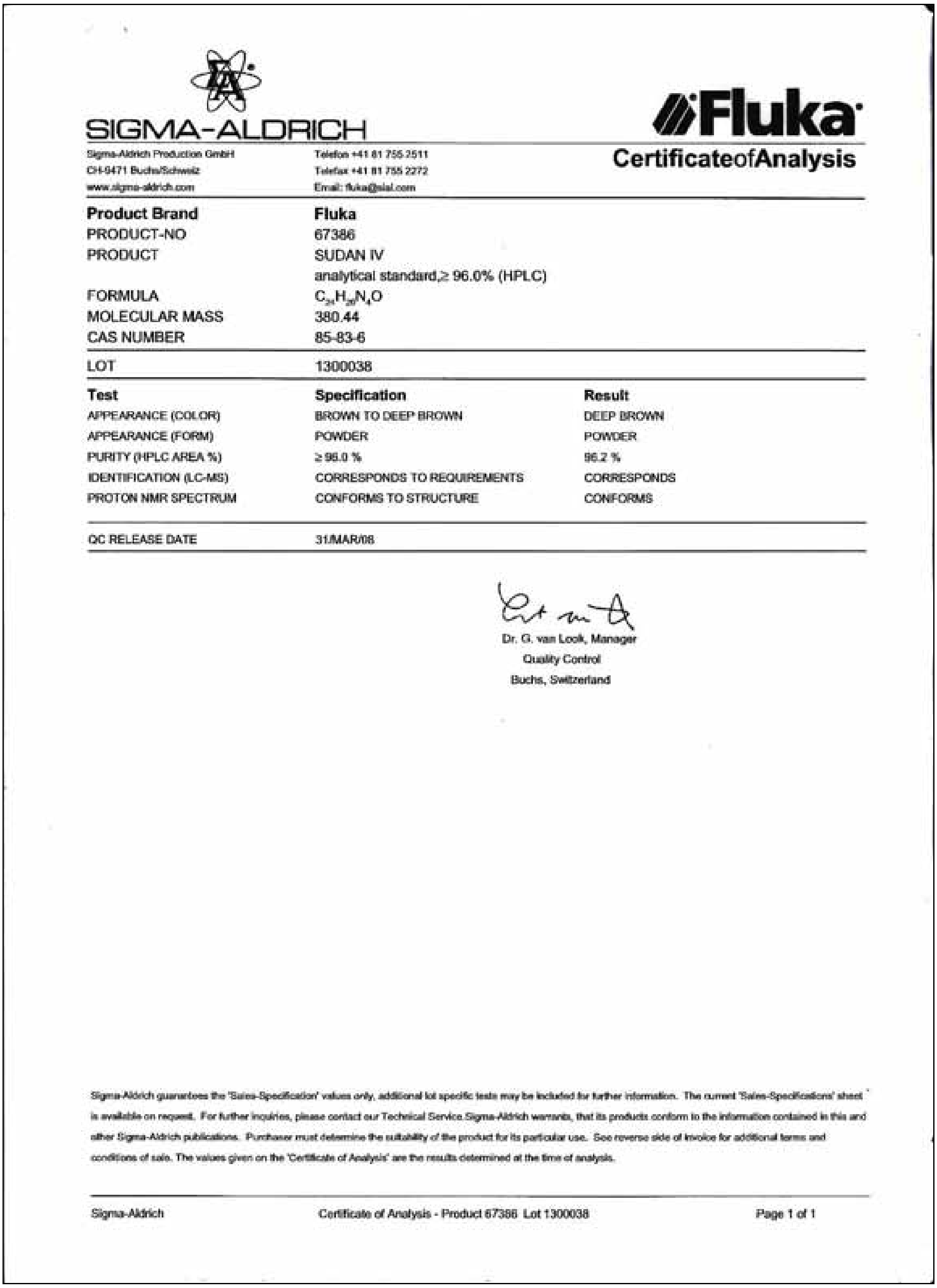
Product Brand	**Fluka**
PRODUCT-NO	68562
PRODUCT	SUDAN III ANALYTICAL STANDARD
FORMULA	$C_{22}H_{16}N_4O$
MOLECULAR MASS	352.39
CAS NUMBER	85-86-9
LOT	1300034

Test	Specification	Result
APPEARANCE (COLOR)	RED/BROWNISH-RED TO DEEP RED/BROWNISH-RED	DEEP BROWNISH-RED
APPEARANCE (FORM)	POWDER TO FINE CRYSTALS	POWDER
PURITY (HPLC AREA %)	≥ 96.0 %	96.0 %
IDENTIFICATION (LC-MS)	CORRESPONDS TO REQUIREMENTS	CORRESPONDS
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

QC RELEASE DATE 31/MAR/08

Dr. G. van Look, Manager
Quality Control
Buchs, Switzerland

Sigma-Aldrich Certificate of Analysis - Product 68562 Lot 1300034 Page 1 of 1



SIGMA-ALDRICH

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Fluka

Certificate of Analysis

Product Brand	**Fluka**
PRODUCT-NO	67386
PRODUCT	SUDAN IV analytical standard,≥ 96.0% (HPLC)
FORMULA	$C_{24}H_{20}N_4O$
MOLECULAR MASS	380.44
CAS NUMBER	85-83-6
LOT	1300038

Test	**Specification**	**Result**
APPEARANCE (COLOR)	BROWN TO DEEP BROWN	DEEP BROWN
APPEARANCE (FORM)	POWDER	POWDER
PURITY (HPLC AREA %)	≥ 96.0 %	96.2 %
IDENTIFICATION (LC-MS)	CORRESPONDS TO REQUIREMENTS	CORRESPONDS
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

QC RELEASE DATE 31/MAR/08

Dr. G. van Look, Manager
Quality Control
Buchs, Switzerland

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