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**Oil of lemon [*Citrus limon* (L.) Burm. f.],  
obtained by expression**

*Huile essentielle de citron [Citrus limon (L.) Burm. f.], obtenue par  
expression*

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## Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 855 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

This second edition cancels and replaces the first edition (ISO 855:1981), which has been technically revised.

Together with the revised versions of ISO 3519 and ISO 8899, it will also cancel and replace ISO 7611:1985.



# Oil of lemon [*Citrus limon* (L.) Burm. f.], obtained by expression

## 1 Scope

This International Standard specifies certain characteristics of the oil of lemon [*Citrus limon* (L.) Burm. f.], obtained by expression, in order to facilitate assessment of its quality.

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TR 210, *Essential oils — General rules for packaging, conditioning and storage*

ISO/TR 211, *Essential oils — General rules for labelling and marking of containers*

ISO 212, *Essential oils — Sampling*

ISO 279, *Essential oils — Determination of relative density at 20 °C — Reference method*

ISO 280, *Essential oils — Determination of refractive index*

ISO 592, *Essential oils — Determination of optical rotation*

ISO 875, *Essential oils — Evaluation of miscibility in ethanol*

ISO 1242, *Essential oils — Determination of acid value*

ISO 1271, *Essential oils — Determination of carbonyl value — Free hydroxylamine method*

ISO 4715, *Essential oils — Quantitative evaluation of residue on evaporation*

ISO 4735, *Oils of Citrus — Determination of CD value by ultraviolet spectrometric analysis*

ISO 11024-1, *Essential oils — General guidance on chromatographic profiles — Part 1: Preparation of chromatographic profiles for presentation in standards*

ISO 11024-2, *Essential oils — General guidance on chromatographic profiles — Part 2: Utilization of chromatographic profiles of samples of essential oils*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

### 3.1

#### oil of lemon

essential oil obtained by expression, without the aid of heat and with or without previous separation of the pulp and the peel, from the fresh fruit of *Citrus limon* (L.) Burm. f., of the Rutaceae family, growing mainly in Argentina, Brazil, Cyprus, Italy, Ivory Coast, Spain, South Africa and United States.

NOTE For information on the CAS number, see ISO/TR 21092.

## 4 Requirements

### 4.1 Appearance

| American type                                                             |             | Mediterranean type |       | Equatorial          |
|---------------------------------------------------------------------------|-------------|--------------------|-------|---------------------|
| Coastal type                                                              | Desert type | Spain              | Italy | Ivory Coast, Brazil |
| Mobile, clear liquid, which may become cloudy by lowering the temperature |             |                    |       |                     |

### 4.2 Colour

| American type                  |             | Mediterranean type |       | Equatorial          |
|--------------------------------|-------------|--------------------|-------|---------------------|
| Coastal type                   | Desert type | Spain              | Italy | Ivory Coast, Brazil |
| From pale yellow to dark green |             |                    |       |                     |

### 4.3 Odour

| American type                          |             | Mediterranean type |       | Equatorial          |
|----------------------------------------|-------------|--------------------|-------|---------------------|
| Coastal type                           | Desert type | Spain              | Italy | Ivory Coast, Brazil |
| Characteristic of fresh lemon pericarp |             |                    |       |                     |

### 4.4 Relative density, at 20 °C, $d_{20}^{20}$

| American type |       |             |       | Mediterranean type |       |       |       | Equatorial          |       |
|---------------|-------|-------------|-------|--------------------|-------|-------|-------|---------------------|-------|
| Coastal type  |       | Desert type |       | Spain              |       | Italy |       | Ivory Coast, Brazil |       |
| min.          | max.  | min.        | max.  | min.               | max.  | min.  | max.  | min.                | max.  |
| 0,851         | 0,857 | 0,849       | 0,854 | 0,849              | 0,858 | 0,850 | 0,858 | 0,845               | 0,854 |

### 4.5 Refractive index at 20 °C

| American type |         |             |         | Mediterranean type |         |         |         | Equatorial          |         |
|---------------|---------|-------------|---------|--------------------|---------|---------|---------|---------------------|---------|
| Coastal type  |         | Desert type |         | Spain              |         | Italy   |         | Ivory Coast, Brazil |         |
| min.          | max.    | min.        | max.    | min.               | max.    | min.    | max.    | min.                | max.    |
| 1,473 0       | 1,476 0 | 1,473 0     | 1,476 0 | 1,473 0            | 1,476 0 | 1,473 0 | 1,476 0 | 1,473 0             | 1,479 0 |

## 4.6 Optical rotation at 20 °C

| American type         |                       | Mediterranean type    |                       | Equatorial            |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Coastal type          | Desert type           | Spain                 | Italy                 | Ivory Coast, Brazil   |
| Between +57° and +66° | Between +67° and +78° | Between +57° and +66° | Between +57° and +66° | Between +57° and +70° |

## 4.7 Residue on evaporation

| American type |      |             |      | Mediterranean type |      |       |      | Equatorial          |      |
|---------------|------|-------------|------|--------------------|------|-------|------|---------------------|------|
| Coastal type  |      | Desert type |      | Spain              |      | Italy |      | Ivory Coast, Brazil |      |
| min.          | max. | min.        | max. | min.               | max. | min.  | max. | min.                | max. |
| 1,75          | 3,90 | n.a.        | n.a. | 1,50               | 3,90 | 1,50  | 3,90 | 1,50                | 4,00 |

## 4.8 Acid value (max.)

| American type |             | Mediterranean type |       | Equatorial          |
|---------------|-------------|--------------------|-------|---------------------|
| Coastal type  | Desert type | Spain              | Italy | Ivory Coast, Brazil |
| 2             | 2           | 2                  | 2     | 2                   |

## 4.9 Carbonyl value

| American type |      |             |      | Mediterranean type |      |       |      | Equatorial          |      |
|---------------|------|-------------|------|--------------------|------|-------|------|---------------------|------|
| Coastal type  |      | Desert type |      | Spain              |      | Italy |      | Ivory Coast, Brazil |      |
| min.          | max. | min.        | max. | min.               | max. | min.  | max. | min.                | max. |
| 8,0           | 14,0 | 6,25        | 12,0 | 11,0               | 17,0 | 11,0  | 17,0 | 6,0                 | 17,0 |

## 4.10 CD value

| American type |      |             |      | Mediterranean type |      |       |      | Equatorial          |      |
|---------------|------|-------------|------|--------------------|------|-------|------|---------------------|------|
| Coastal type  |      | Desert type |      | Spain              |      | Italy |      | Ivory Coast, Brazil |      |
| min.          | max. | min.        | max. | min.               | max. | min.  | max. | min.                | max. |
| 0,20          | n.a. | 0,20        | n.a. | 0,40               | 0,90 | 0,45  | 0,90 | 0,20                | 0,96 |

#### 4.11 Chromatographic profile

Analysis of the essential oil shall be carried out by gas chromatography. In the chromatogram obtained, the representative and characteristics components shown in Table 1 shall be identified. The proportions of these components, indicated by the integrator, shall be as shown in Table 1. This constitutes the chromatographic profile of the essential oil.

#### 4.12 Flashpoint

Information on the flashpoint is given in Annex B.

### 5 Sampling

See ISO 212.

Minimum volume of test sample: 25 ml.

NOTE This volume allows each of the tests specified in this International Standard to be carried out at least once.

### 6 Test methods

#### 6.1 Relative density at 20 °C, $d_{20}^{20}$

See ISO 279.

#### 6.2 Refractive index at 20 °C

See ISO 280.

#### 6.3 Optical rotation at 20 °C

See ISO 592.

#### 6.4 Residue on evaporation

See ISO 4715.

Test portion: 5 g.

Evaporation time: 5 h.

#### 6.5 Acid value

See ISO 1242.

Test portion: 2 g.

#### 6.6 Carbonyl value

See ISO 1271.

Test portion: 10 g.

Period of standing: 15 min.

Relative molecular mass: 152,23.

#### 6.7 CD value

See ISO 4735.

Point B: 285 nm approximately.

Maximum value (point D): 315 nm approximately.

Point A: 365 nm approximately.

Dilution of 0,25 g of oil in 100 ml of 95 % (volume fraction) ethanol.

#### 6.8 Chromatographic profile

See ISO 11024-1 and ISO 11024-2.

### 7 Packaging, labelling, marking and storage

See ISO/TR 210 and ISO/TR 211.

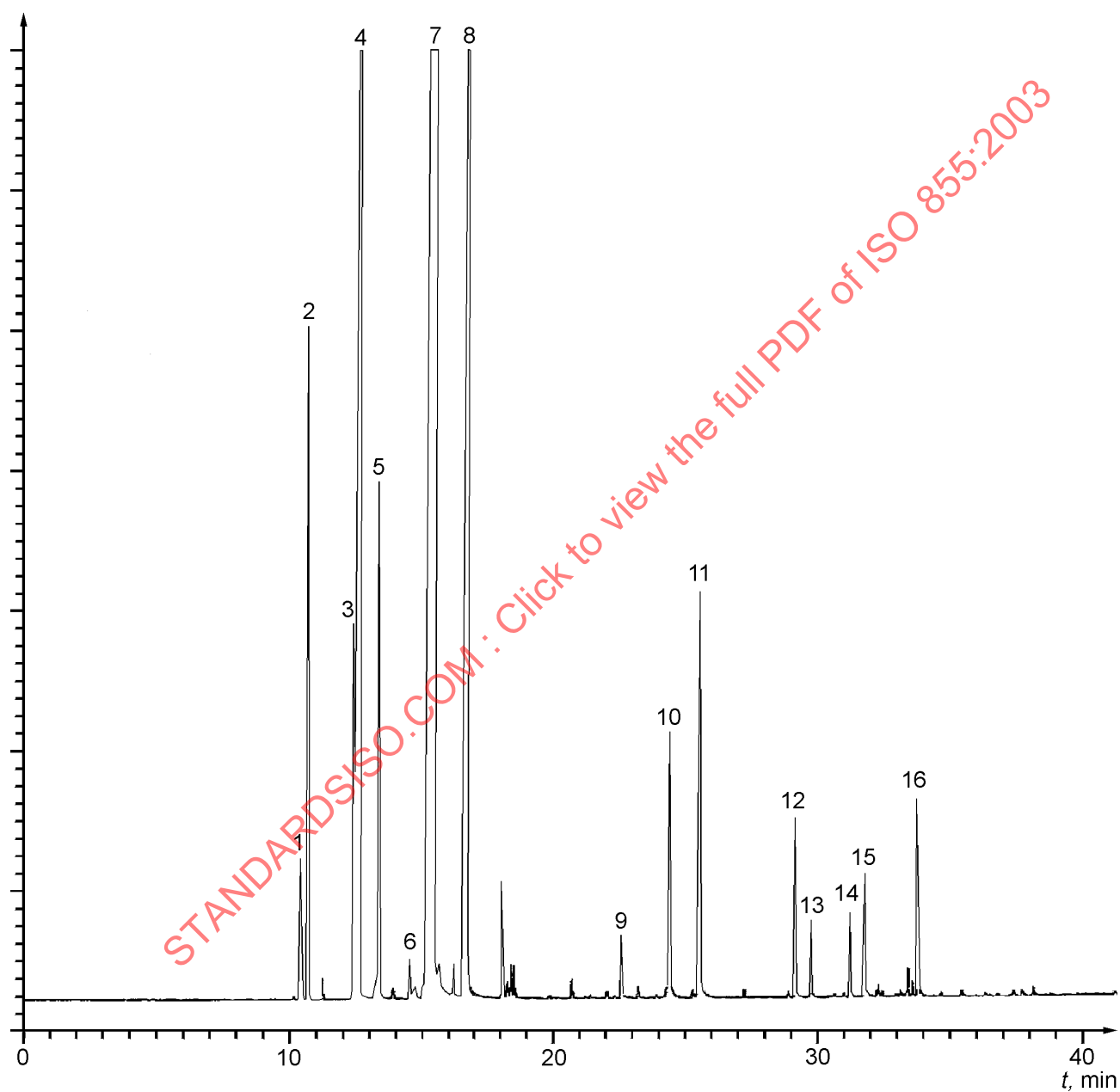
Table 1 — Chromatographic profile

Values in percent

| Components                                                                                                         | American type |      |             |      | Mediterranean type |      |       |      | Equatorial          |      |
|--------------------------------------------------------------------------------------------------------------------|---------------|------|-------------|------|--------------------|------|-------|------|---------------------|------|
|                                                                                                                    | Coastal type  |      | Desert type |      | Spain              |      | Italy |      | Ivory Coast, Brazil |      |
|                                                                                                                    | min.          | max. | min.        | max. | min.               | max. | min.  | max. | min.                | max. |
| $\alpha$ -Thujene                                                                                                  | 0,2           | 0,5  | 0,2         | 0,5  | 0,2                | 0,5  | 0,2   | 0,5  | 0,2                 | 0,5  |
| $\alpha$ -Pinene                                                                                                   | 1,5           | 2,5  | 1,4         | 2,5  | 1,5                | 3,0  | 1,5   | 3,0  | 1,4                 | 3,0  |
| Sabinene                                                                                                           | 1,5           | 2,5  | 1,3         | 2,5  | 1,5                | 3,0  | 1,5   | 3,0  | 1,4                 | 3,0  |
| $\beta$ -Pinene                                                                                                    | 9,0           | 14,0 | 10,0        | 13,0 | 10,0               | 16,5 | 10,0  | 16,5 | 7,0                 | 16,0 |
| <i>p</i> -Cymene                                                                                                   | 0,05          | 0,35 | 0,01        | 0,35 | traces             | 0,40 | 0,05  | 0,35 | 0,05                | 0,35 |
| Limonene <sup>a</sup>                                                                                              | 63,0          | 70,0 | 70,0        | 80,0 | 60,0               | 70,0 | 60,0  | 68,0 | 59,0                | 75,0 |
| $\gamma$ -Terpinene                                                                                                | 8,3           | 9,5  | 6,5         | 8,0  | 8,0                | 12,0 | 8,0   | 12,0 | 6,0                 | 12,0 |
| $\alpha$ -Terpineol                                                                                                | 0,10          | 0,25 | 0,06        | 0,15 | 0,09               | 0,35 | 0,10  | 0,30 | 0,00                | 0,40 |
| Neral                                                                                                              | 0,6           | 0,9  | 0,3         | 0,6  | 0,4                | 1,0  | 0,6   | 1,2  | 0,2                 | 1,2  |
| Geranial                                                                                                           | 1,0           | 2,0  | 0,5         | 0,9  | 0,6                | 2,0  | 0,8   | 2,0  | 0,5                 | 2,0  |
| $\beta$ -Bisabolene                                                                                                | 0,45          | 0,9  | 0,40        | 0,7  | 0,45               | 0,9  | 0,45  | 0,9  | 0,20                | 0,9  |
| Neryl acetate                                                                                                      | 0,35          | 0,60 | 0,30        | 0,50 | 0,30               | 0,60 | 0,20  | 0,50 | 0,10                | 0,50 |
| Geranyl acetate                                                                                                    | 0,20          | 0,50 | 0,10        | 0,30 | 0,20               | 0,65 | 0,30  | 0,65 | traces              | 0,30 |
| NOTE The chromatographic profile is normative, contrary to typical chromatograms given for information in Annex A. |               |      |             |      |                    |      |       |      |                     |      |
| <sup>a</sup> This is regarded as being completely D-limonene by independent chemical and physical analysis.        |               |      |             |      |                    |      |       |      |                     |      |

## Annex A (informative)

### Typical chromatograms of the analysis by gas chromatography of the essential oil of lemon [*Citrus limon* (L.) Burm. f.]



**Peak identification**

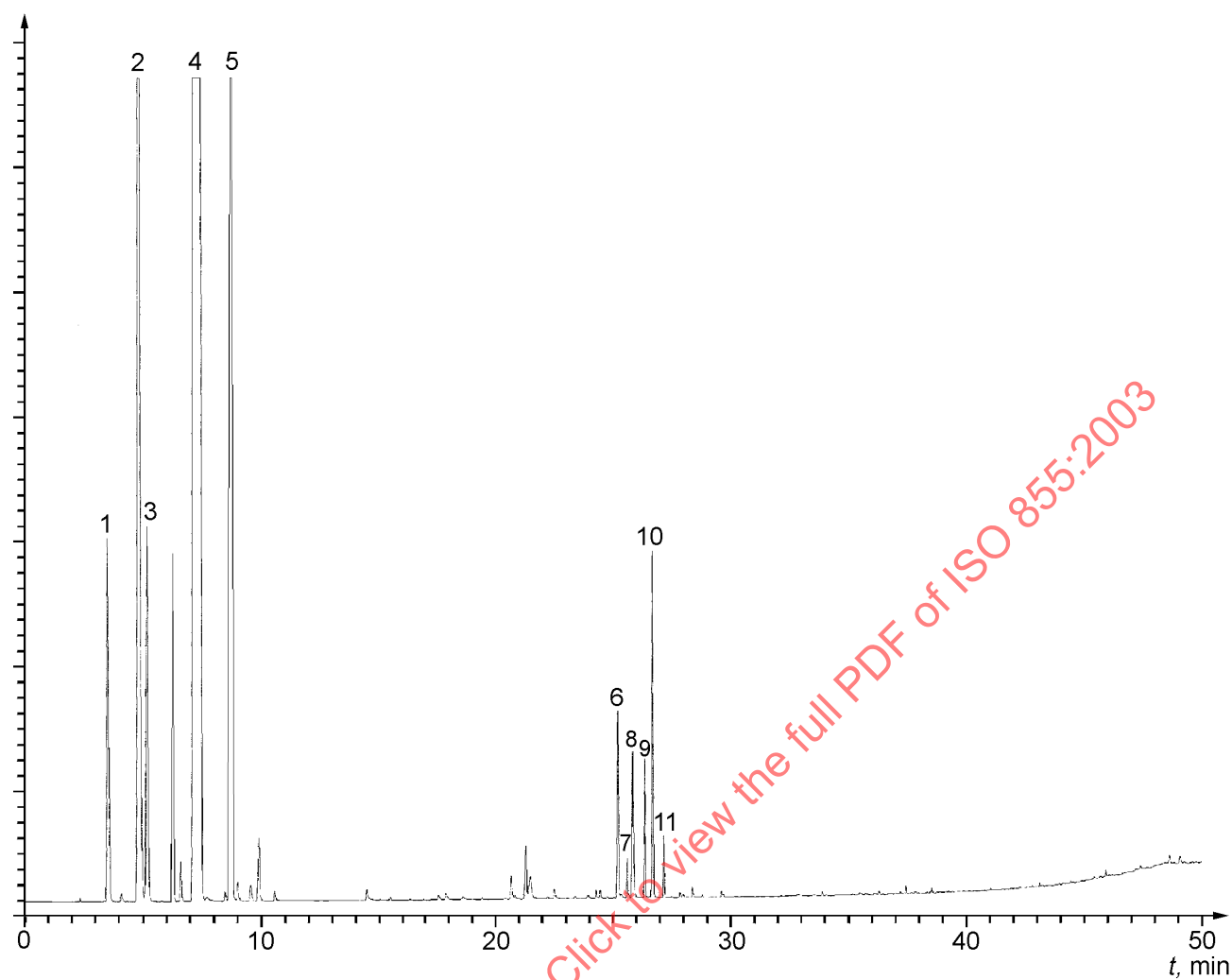
- 1  $\alpha$ -Thujene
- 2  $\alpha$ -Pinene
- 3 Sabinene
- 4  $\beta$ -Pinene
- 5 Myrcene
- 6 *p*-Cymene
- 7 Limonene
- 8  $\gamma$ -Terpinene
- 9  $\alpha$ -Terpineol
- 10 Neral
- 11 Geranial
- 12 Neryl acetate
- 13 Geranyl acetate
- 14  $\beta$ -Caryophyllene
- 15  $\alpha$ -Bergamotene
- 16  $\beta$ -Bisabolene

**Operating conditions**

Column: fused silica capillary; length 30 m; internal diameter 0,25 mm  
 Stationary phase: poly(dimethyl siloxane) (DB-1®)  
 Thickness of film: 0,25  $\mu$ m  
 Oven temperature: temperature programming from 75 °C to 100 °C, at a rate of 5 °C/min and from 100 °C to 220 °C at 6 °C/min  
 Injector temperature: 230 °C  
 Detector temperature: 260 °C  
 Detector: flame ionization type  
 Carrier gas: helium  
 Volume injected: 0,2  $\mu$ l  
 Carrier gas flow rate: 1 ml/min  
 Split ratio: 100/1

**Figure A.1 — Typical chromatogram taken on a non-polar column**

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**Peak identification**

- 1  $\alpha$ -Pinene
- 2  $\beta$ -Pinene
- 3 Sabinene
- 4 Limonene
- 5  $\gamma$ -Terpinene
- 6 Neral
- 7  $\alpha$ -Terpineol
- 8  $\beta$ -Bisabolene
- 9 Neryl acetate
- 10 Geranial
- 11 Geranyl acetate

**Operating conditions**

Column: fused silica capillary; length 30 m; internal diameter 0,25 mm  
 Stationary phase: poly(ethylene glycol) (Carbowax 20 M<sup>®</sup>)  
 Thickness of film: 0,25  $\mu$ m  
 Oven temperature: temperature programming from 75 °C to 100 °C at a rate of 5 °C/min and from 100 °C to 220 °C at 6 °C/min  
 Injector temperature: 230 °C  
 Detector temperature: 260 °C  
 Detector: flame ionization type  
 Carrier gas: helium  
 Volume injected: 0,2  $\mu$ l  
 Carrier gas flow rate: 1 ml/min  
 Split ratio: 100/1

**Figure A.2 — Typical chromatogram taken on a polar column**