

# European Pharmacopoeia Reference Standards for Herbal Drugs and Preparations

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# Content of the presentation

- Definitions and guidelines
- Previous and new policy in monographs on herbal drugs
- Testing
- Examples
- Assignment and use

# Definitions and Guidelines (2)

- General Chapter 5.12.

Reference Standards (adopted in 11/2005)

« Reference standards are also established for the determination of the content of components of herbal drugs and herbal drug preparations. These may be: the active principles themselves, marker constituents used for quantification, or extracts. Reference standards consisting of extracts are established using well-characterised samples of active principles or markers »

# Previous policy

- In case of HPLC assays description of a reagent of minimum purity as “reference substance”. Purity had to be determined by the user.

# **New policy**

- **Previous system was considered insufficient because of:**
  - **Inconsistency with policy applied in monographs for substances for pharmaceutical use**
  - **Assignment to be done by the user**
  - **Quality of the plant material not controlled in a harmonised manner**
  - **Availability and costs of reagents**

# **New policy**

- **In future reference substances with assigned contents will be described in monographs for herbal drugs which may be either:**
  - **the “active principle” or a marker substance or**
  - **a standardized extract**

**The choice depends on availability, price, stability**

# Active ingredients or marker substances

**Boldine CRS - Monograph boldo leaf**

**Coumarin CRS - Monograph melilot**

**Ruscogenins CRS - Monograph butcher's broom**

**Chlorogenic acid CRS – Monograph nettle leaf, purple coneflower root and herb**

**Verbenalin CRS - monograph verbena herb**

# Active ingredients or marker substances

- Salicin CRS - monograph willow bark
- Capsaicin CRS - monographs for standardized capsicum tincture, capsicum oleoresin
- Nonivamide CRS – see capsaicin
- Harpagoside - monograph Devil's claw root
- Oleuropein – monograph olive dry extract
- Aescin – monograph horse chestnut dry extract



# Active ingredients or marker substances

- Rhein CRS – draft monograph rhubarb
- Rosmarinic acid – melissa leaf and extract, peppermint leaf and extract

# Establishment of active ingredients or markers

- Structure elucidation by IR, NMR, MS
- Purity determination by HPLC
- Determination of water and solvents and/or LOD/TGA
- Inorganic impurities
- DSC or other absolute methods such as titration

# Establishment of active ingredients or markers

- Content assignment on the “as is” basis  
 $100 - (\text{water} + \text{solvents}) \times \text{chromatographic purity} / 100$
- Lyophilized substance (verbenalin):  
assignment on a mg/vial basis (ex. 0.97 mg of verbenalin/vial for verbenalin CRS 1)

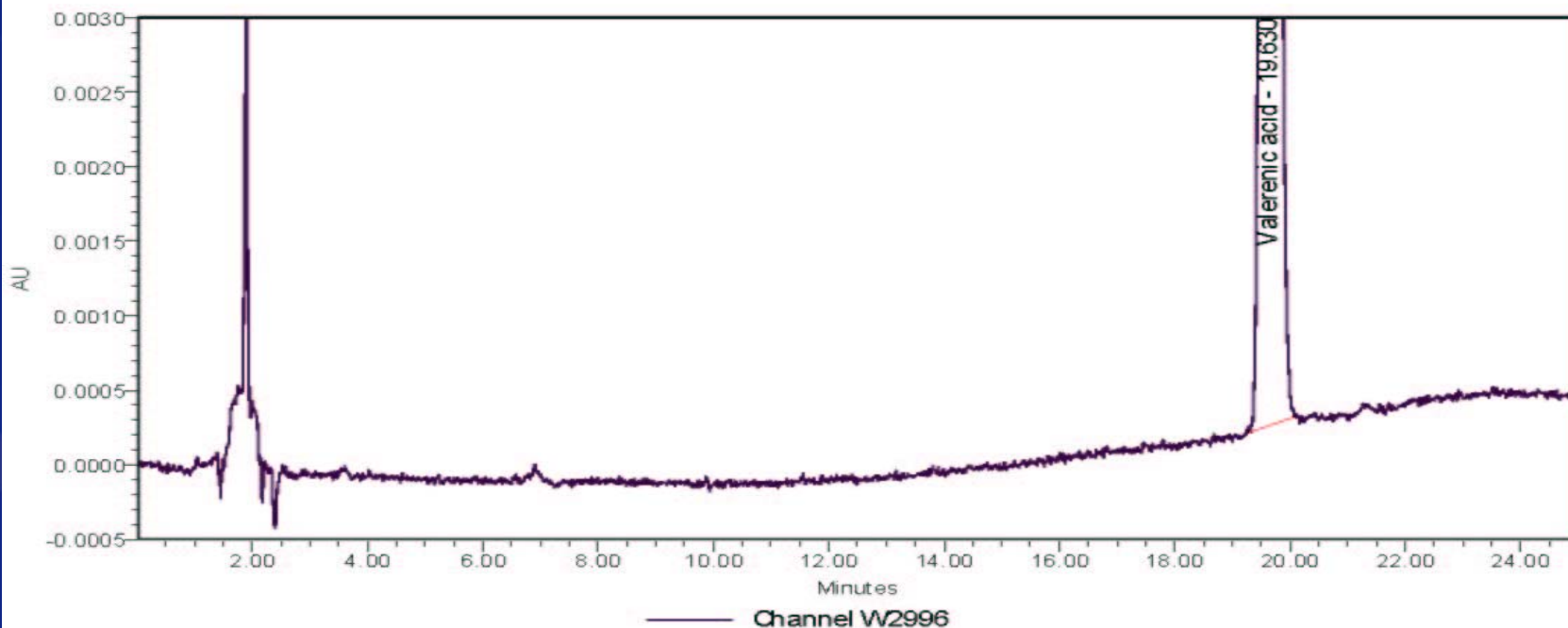
# Standardized extracts

Option chosen when the “active principle” or marker substance are not available in sufficient amounts:

- Milk thistle standardized dry extract
- Ivy leaf standardized tincture
- Valerian standardized dry extract
- Agnus castus fruit standardized dry extract

# Standardized extracts

Example: Valerian standardized extract and Valerenic acid

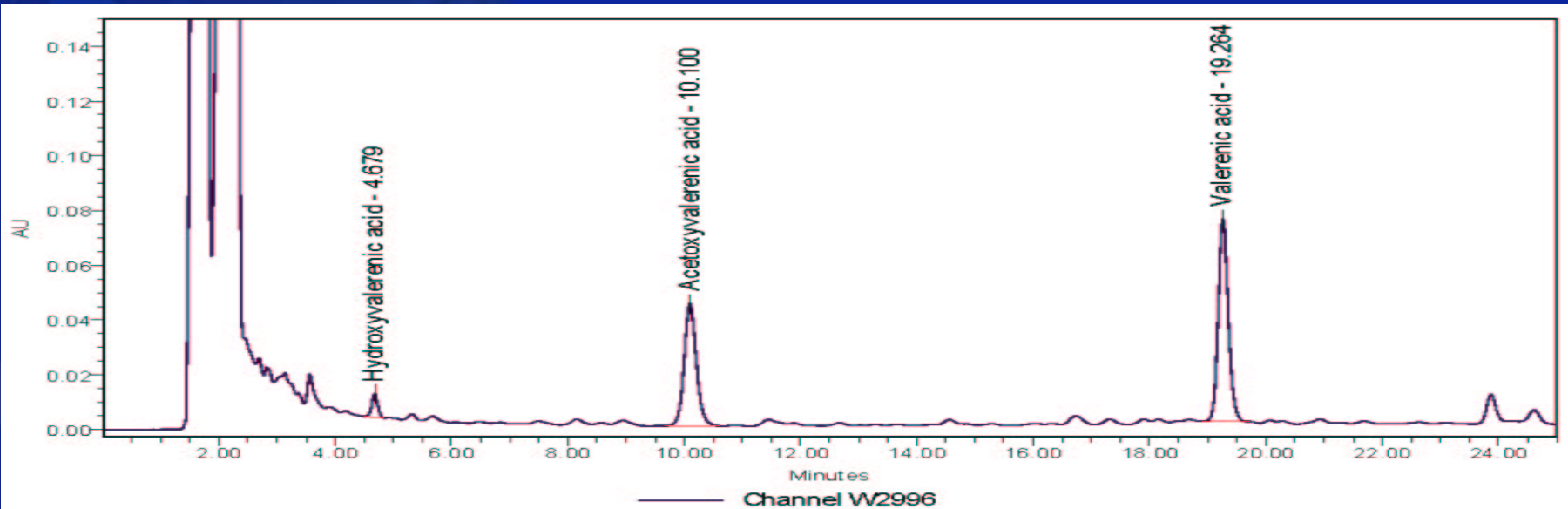


Peak Results

	<i>Name</i>	<i>RT</i>	<i>Area</i>	<i>%Area</i>	<i>Height</i>	<i>Int Type</i>	<i>SN</i>
1	Valerenic acid	19.630	1101746	100.00	92174	BB	1825

# Standardized extracts

Example: *Valerian standardized extract* and Valerenic acid

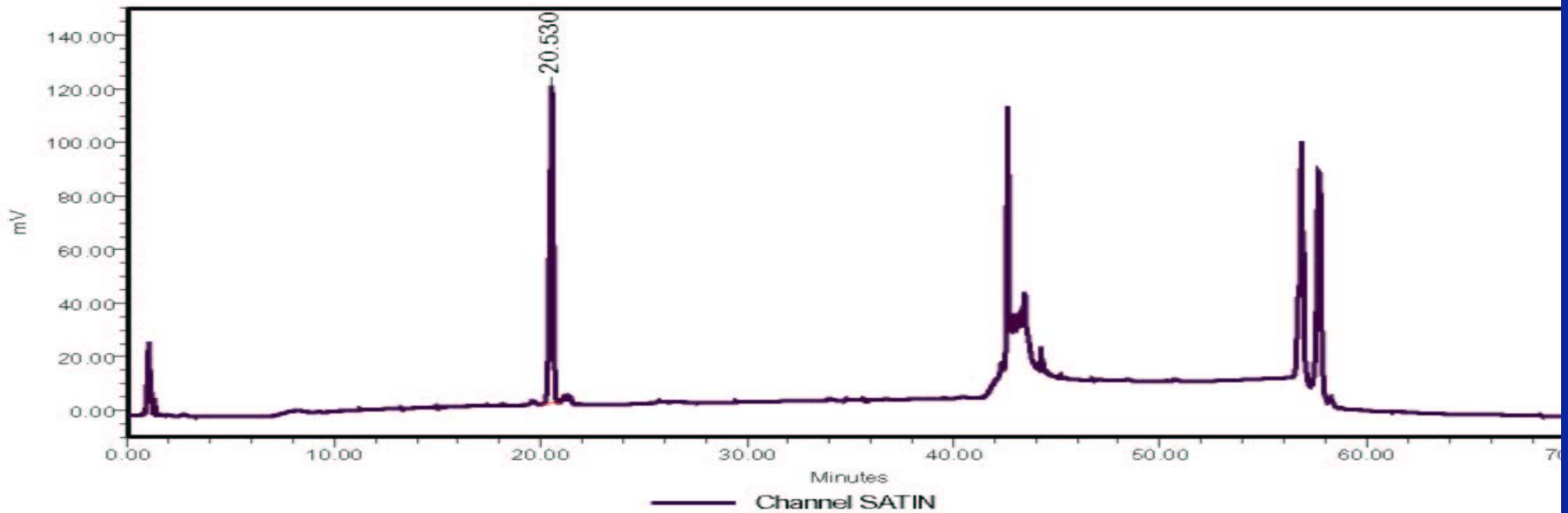


Peak Results

	<b>Name</b>	<b>RT</b>	<b>RT Ratio</b>	<b>Area</b>	<b>%Area</b>	<b>Height</b>	<b>Int Type</b>	<b>SN</b>
1	Hydroxyvalerenic acid	4.679	0.2	59823	3.73	8593	BB	170
2	Acetoxyvalerenic acid	10.100	0.5	649611	40.53	44726	BB	886
3	Valerenic acid	19.264		893229	55.73	74211	bB	1470

# Standardized extracts

Example: *Hederacoside* and Hedera helix tincture

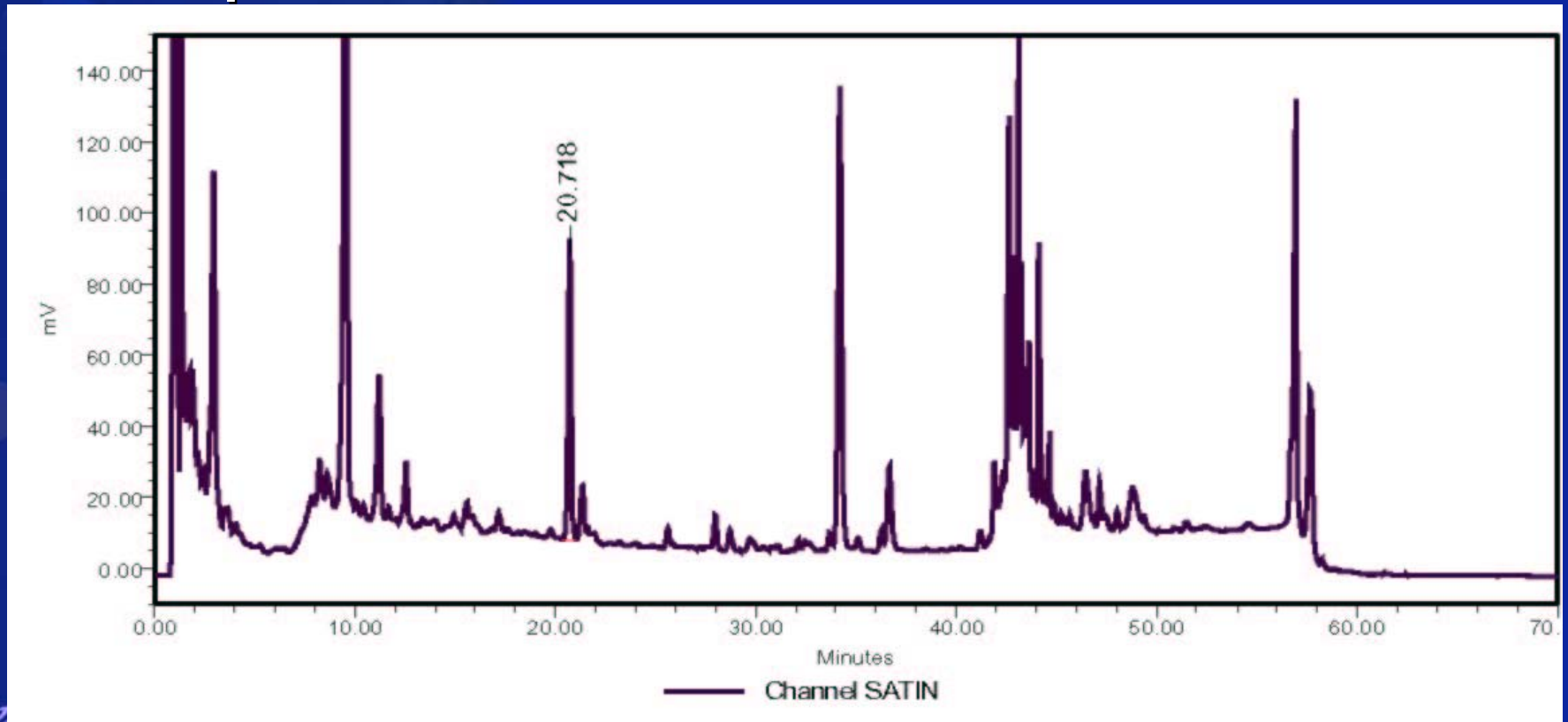


Peak Results

	Name	RT	Area	% Area	Height	Int Type
1	Hederacoside C	20.530	1395017	100.00	118356	Eb

# Standardized extracts

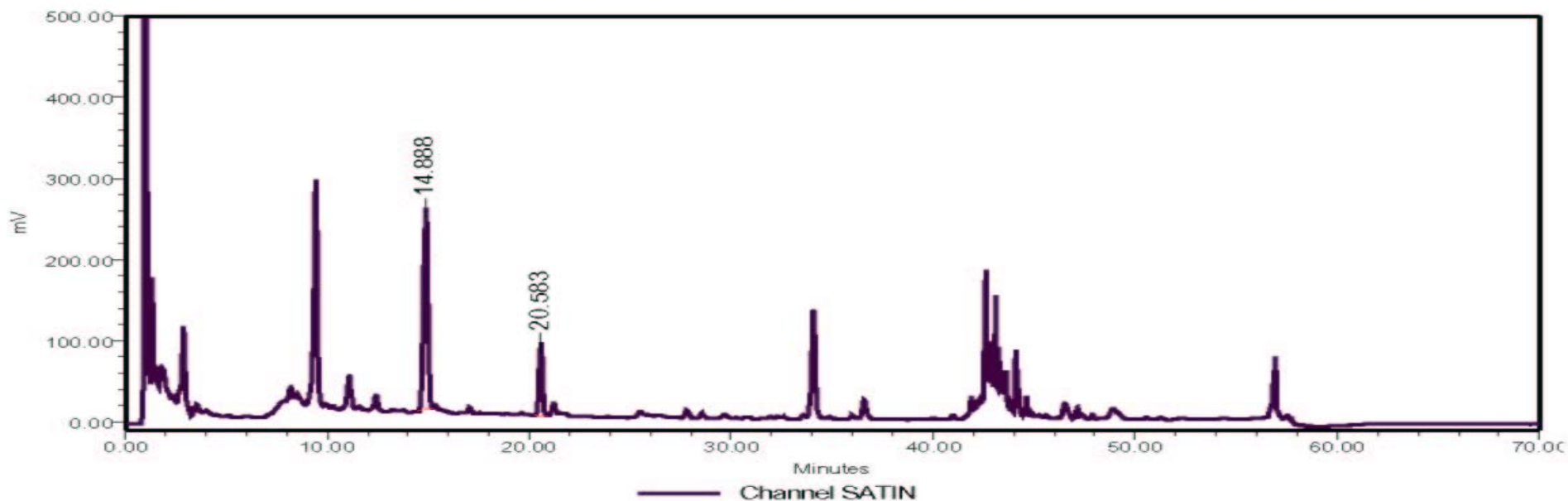
Example: Hederacoside and *Hedera helix* tincture





# Standardized extracts (monitoring)

Example: Hedera helix tincture (addition of ethylparaben)



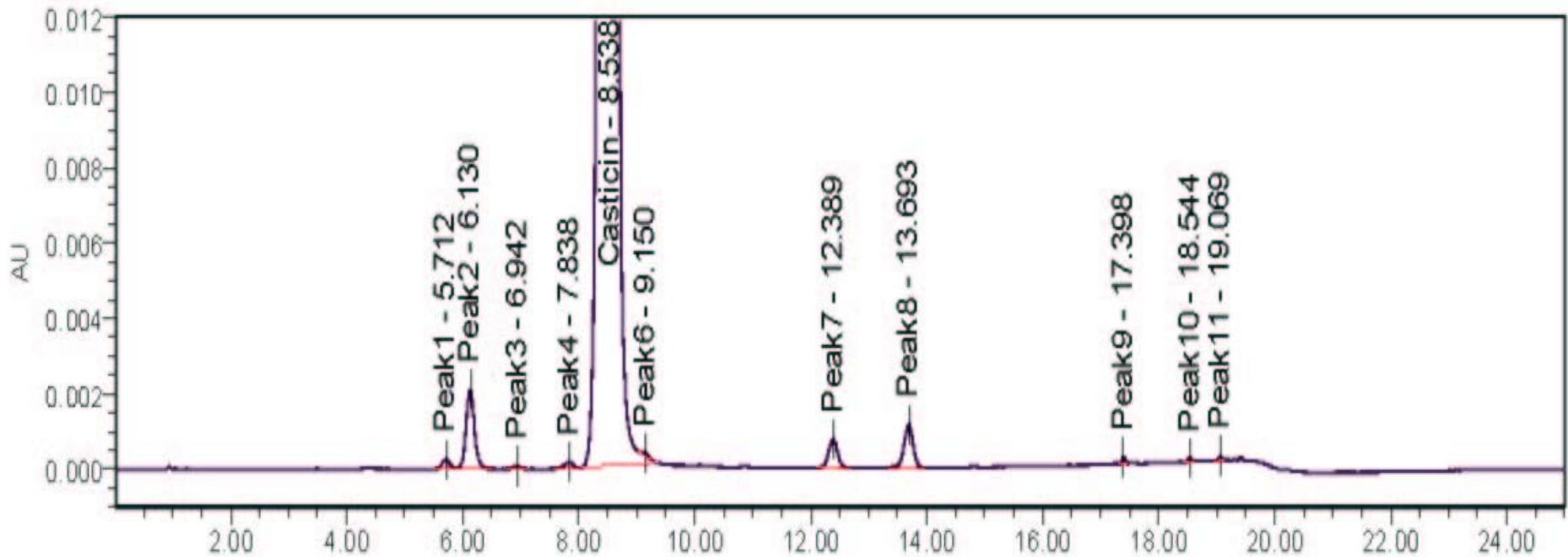
Peak Results

	<b>Name</b>	<b>RT</b>	<b>Area</b>	<b>% Area</b>	<b>Height</b>	<b>Int Type</b>
1	ethyl-paraben	14.888	3596704	77.86	247809	Vb
2	Hederacoside C	20.583	1022516	22.14	89613	BB

# Standardized extracts

Example: Agnus Castus dry extract

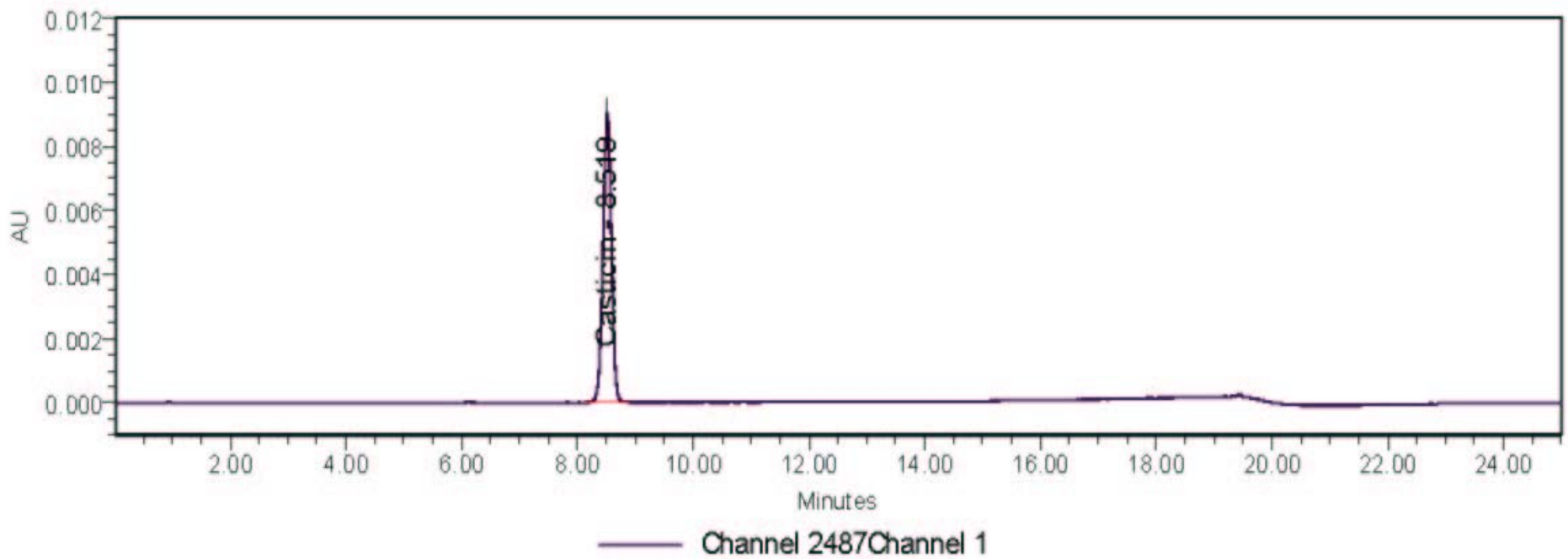
Casticin test solution



# Standardized extracts

Example: Agnus Castus dry extract

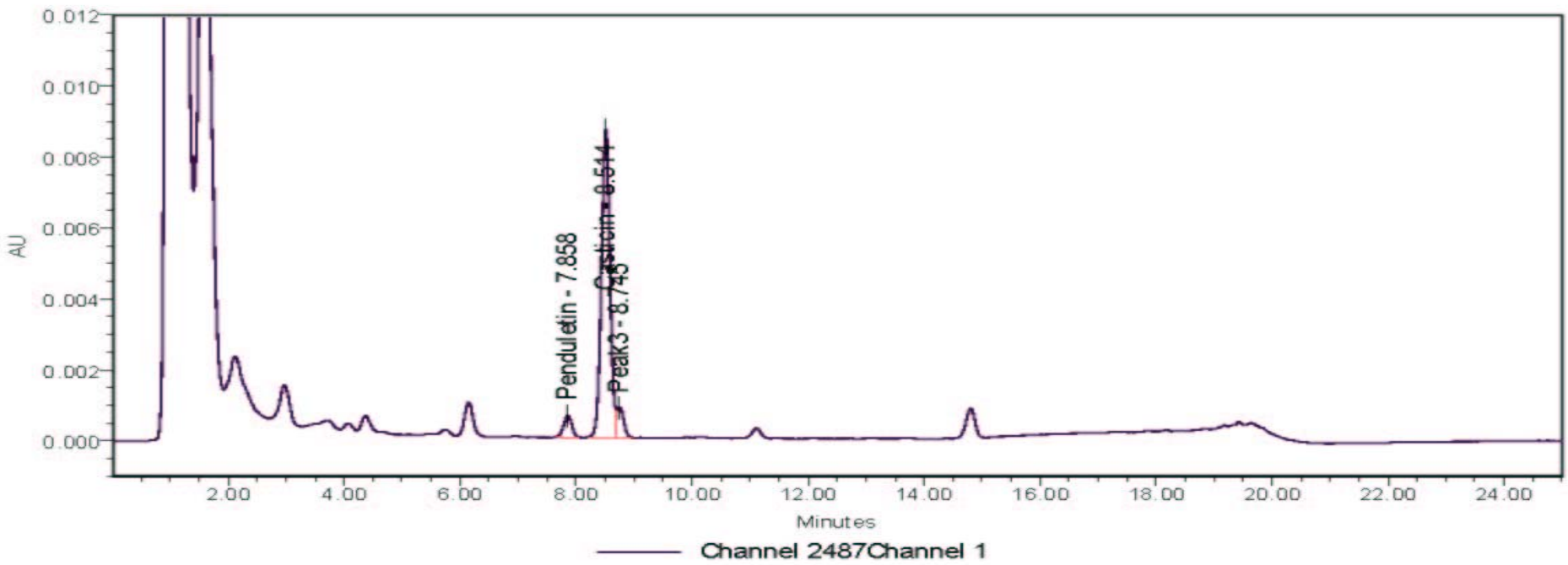
Casticin reference solution



# Standardized extracts

Example: Agnus Castus dry extract

Test solution



# Use of a marker as CRS

- Problem: neither active principle nor extract are available:

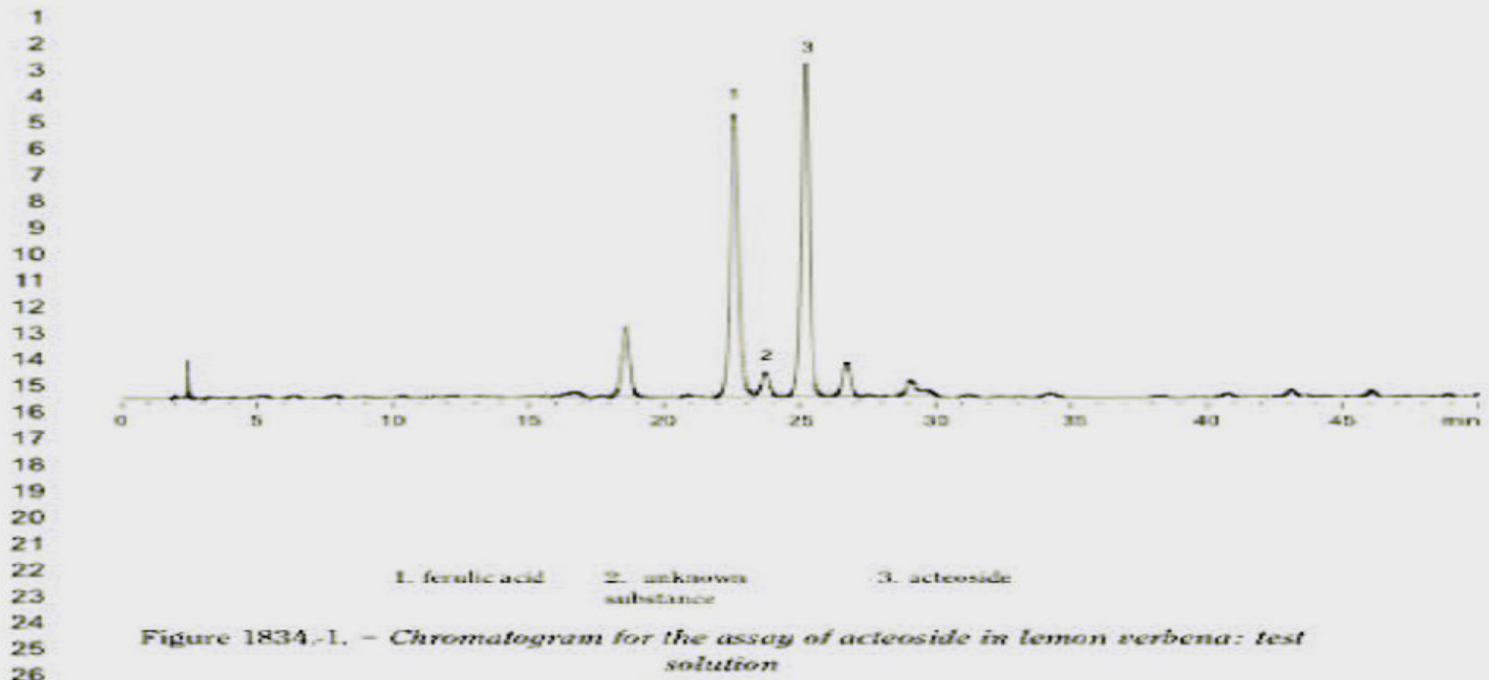
**Example: Monograph for Lemon verbena leaf**

Definition: Content minimum 2.5 % of acteoside

HPLC assay: resolution requirement of 3.5 for the separation between acteoside and internal standard (ferulic acid R)

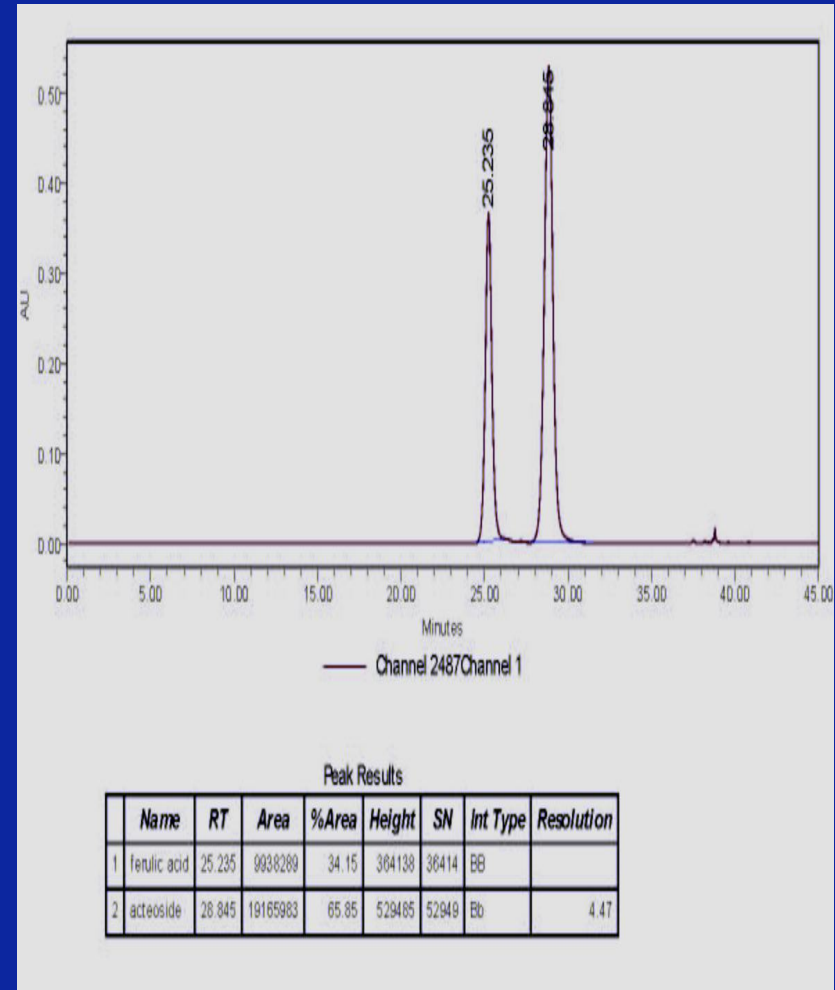
# Use of a marker as CRS

## Test solution with addition of ferulic acid



# Use of a marker as CRS

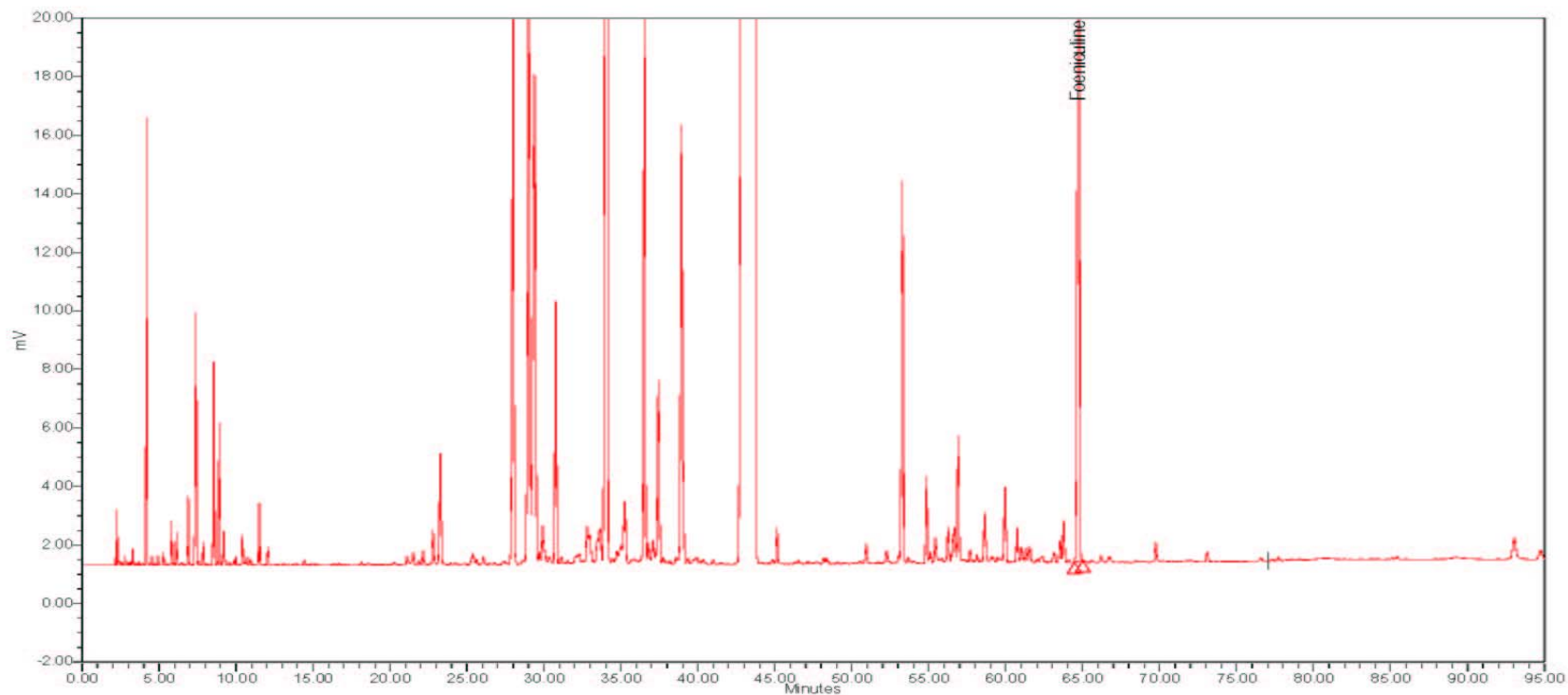
- Solution:
  - Determination of response factor of acteoside/ferulic acid
  - Use of ferulic acid as CRS with assigned content



# CRS in the test for related substances: Qualitative use for peak identification Monograph Anise oil, GC-test for foeniculin

*EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES*

*Foeniculin for peak identification CRS*





**Thank you for your attention and  
patience**