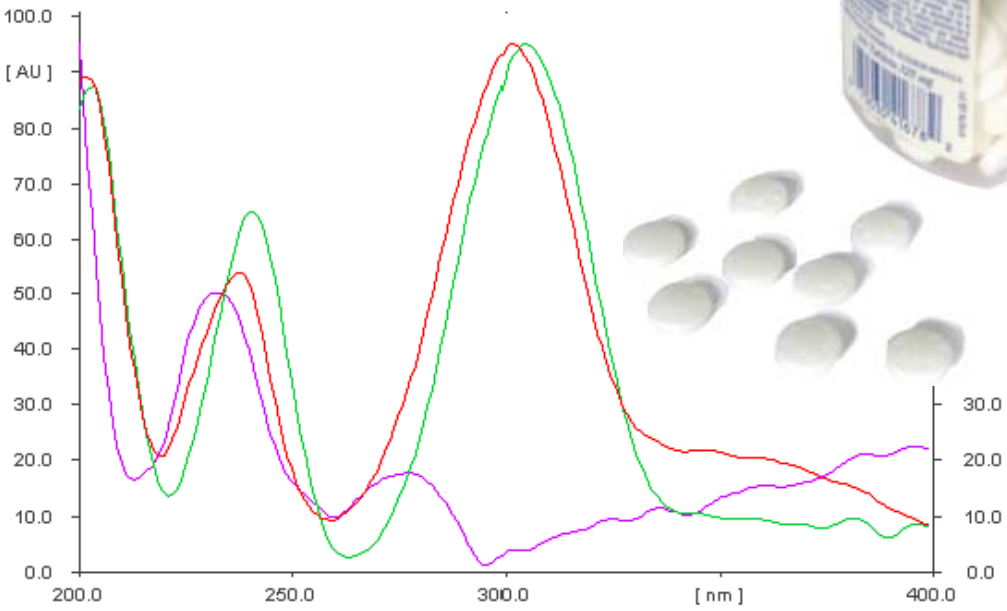
Quantitation of acetylsalicylic acid, salicylic acid, and salicyl amide by HPTLC A-14.4

Key words

- Quantitative HPTLC
- Densitometry
- Spectral evaluation
- Analgesic
- Acetylsalicylic acid
- Salicylic acid
- Salicyl amide



Scope

Salicylic acid derivatives are contained in antipyretic, anti-inflammatory, and analgesic drugs. Salicylic acid may be present as degradation product. It is also used as topical keratolytic.

The method is suitable for quantitation of actives in pharmaceutical products, detection of impurities as result of degradation and identification of unknown substances. Identification is based on Rf-values and spectral parameters, quantitation is performed by densitometry at 200 nm.

Required or recomended CAMAG devices

Automatic TLC Sampler III or Automatic TLC Sampler 4 or Linomat IV / or 5

Twin Trough Chamber 20 x10 cm / 10x10 cm

DigiStore

TLC Scanner 3 and winCATS software

Chemicals

Cyclohexane – chloroform - acetic acid – acetylsalicylic acid – salicylic acid – salicyl amide

HPTLC plates silica gel 60 F_{254} (Merck), 20x10 or 10x10 cm. The plate is pre-washed by development with developing solvent to upper edge and dried at 120°C for 60 min.

Sample

The sample solution is prepared to have approximately the same concentration as the standard solution B.

Sample application

5 mL of samples and 3, 4, 5, 6, and 7 mL of the standard solution are applied with the CAMAG Automatic TLC Sampler 4 as 6 mm bands, 8 mm from the lower edge of the plate, and at least 10 mm from the sides.

Standards

Solution A: In a 100 mL volumetric flask 50 mg acetylsalicylic acid, 25 mg salicylic acid, and 25 mg salicyl amide are weighed, dissolved in and filled to the mark with ethanol-hexane 1:1.

Solution B: 2 mL of solution A are diluted to 20 mL with ethanol-hexane 1:1.

1 mL solution B contains: 50 ng acetylsalicylic acid, 25 ng salicylic acid, 25 ng salicyl amide

Chromatography

Mobile phase: Cyclohexane, chloroform, acetic acid (60:5:5)

Development: 10/5 mL developing solvent are placed in each trough of a 20x10/10x10 cm Twin Trough Chamber, containing a filter paper on one side. The lid is closed and the chamber allowed to saturate for 30 min. Developing distance is 7 cm measured from lower edge of plate. The plate is dried in a stream of cold air.

Documentation (optional)

With CAMAG DigiStore under 254 nm and 366 nm.

Densitometry

With CAMAG TLC Scanner 3 and winCATS software, absorbance measurement at 200 nm (D_2 lamp).

Note: Salicyl amide and acetylsalicylic acid can also be measured at 304 nm (absorbance). At this wavelength, salicylic acid shows practically no absorption (see spectra below).

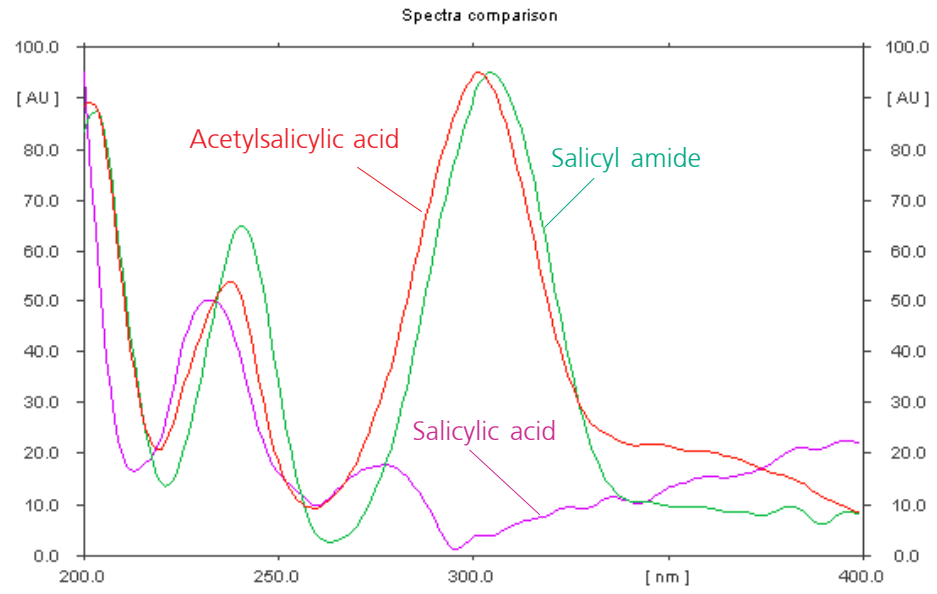
Results screening:

UV 254 nm: very faint zones are seen (no image):

Salicyl amide	Rf = 0.06
Salicylic acid	Rf = 0.12
Acetylsalicylic acid	Rf = 0.20

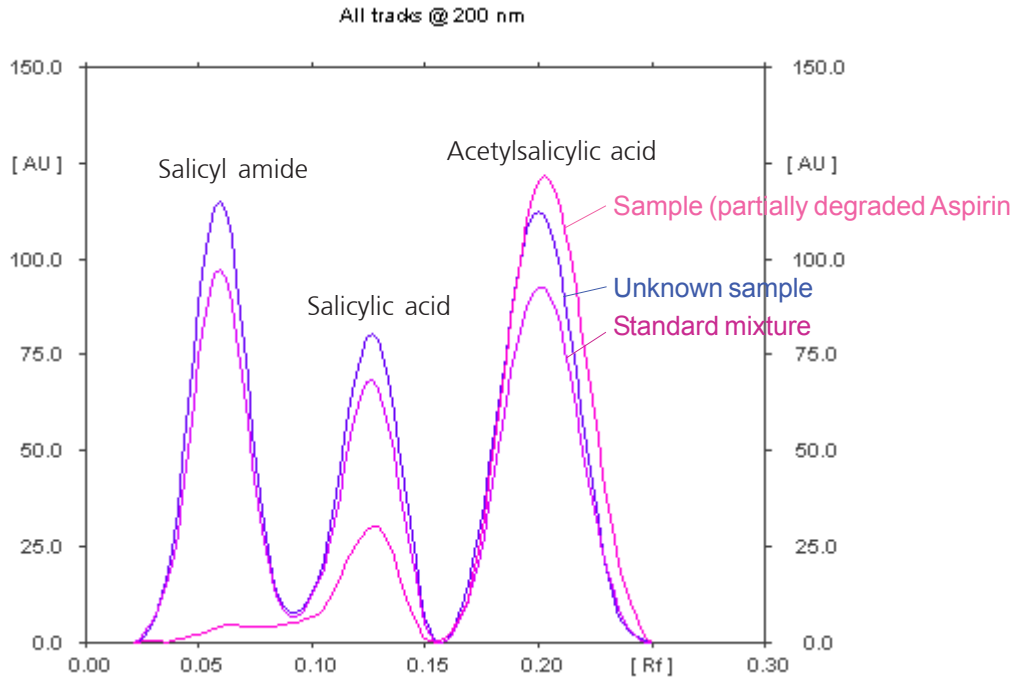
UV 366 nm: Salicyl amide and acetylsalicylic acid give blue fluorescence, salicylic acid is not detected.

Image under UV 366 nm

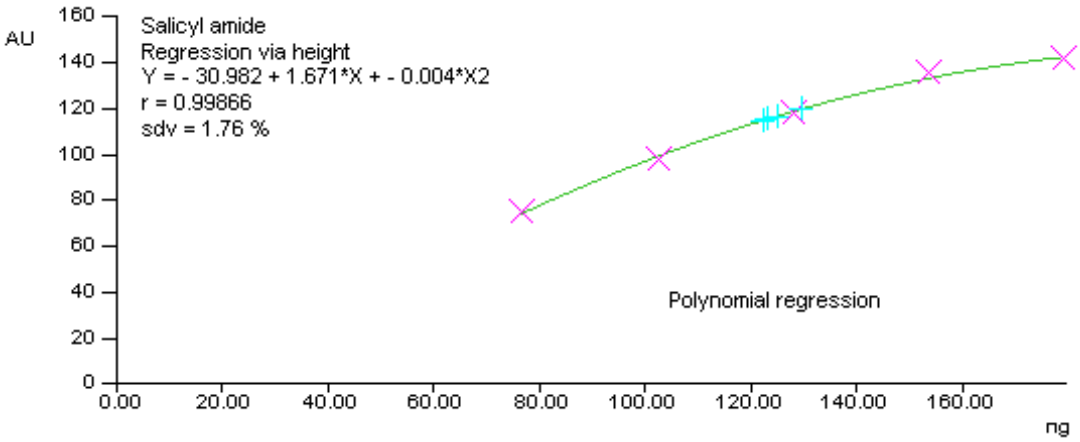


UV spectra

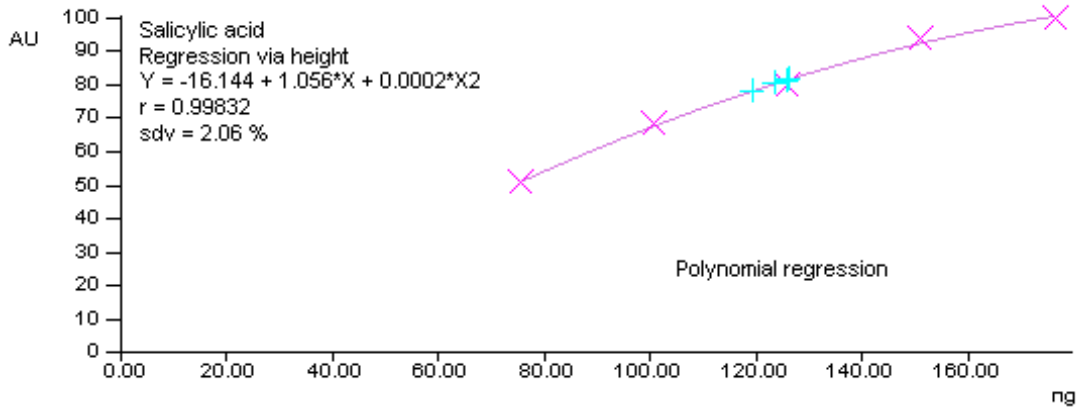
Results densitometry:



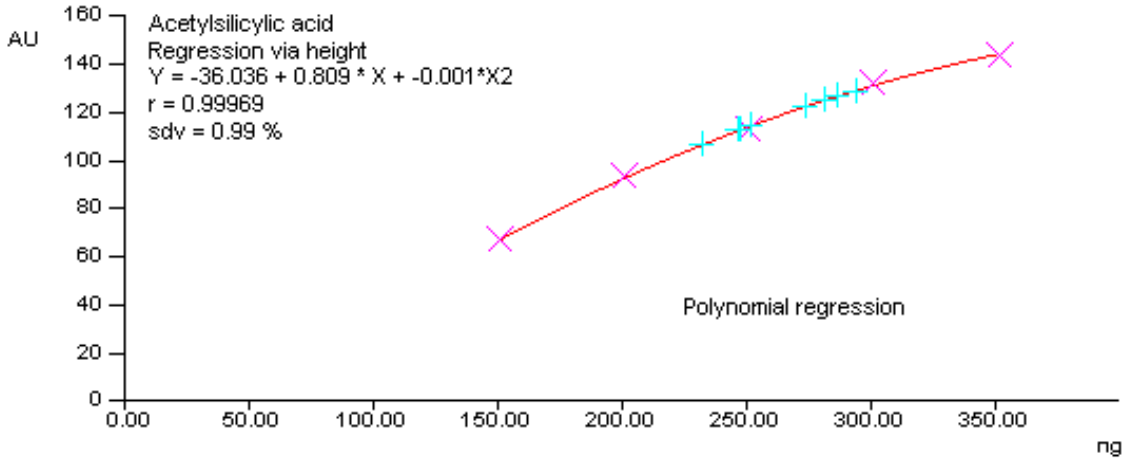
Overlay of densitograms (analog curves), absorbance at 200 nm



Calibration for salicyl amide, 75-175 ng (peak height)



Calibration for salicylic acid, 75-175 ng (peak height)



Calibration for acetylsalicylic acid, 150-350 ng (peak height)

Note: The presented results are to be regarded as examples only!