Is there a fine future for HPTLC in the modern API plants? Actual experiences

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Sanofi-aventis at a glance

- **Sanofi-aventis is a world leader in the pharmaceutical industry, n° 1 in Europe and in BRIC-M***
  - **28,052 million euros consolidated sales in 2007**
  - **A world leader in high-growth therapeutic areas**
    - **n°1 in thrombosis**
    - **n°2 in vaccines**
    - **n°2 in diabetes**
    - **n°2 in oncology**

* Brazil, Russia, India, China and Mexico – source IMS

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Sanofi-aventis at a glance

Sanofi-aventis' growth is based on a regional approach to markets…

- **Well balanced net sales**
  - Europe 44 %
  - United States 34 %
  - Other countries 22 %
- **Nearly 100,000 employees**
  - 55,000 in Europe,
  - 16,000 in the United States,
  - 29,000 in other countries

…and offering a full range of medicines and vaccines.

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A complete portfolio

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Sanofi-aventis Neuville-sur-Saône site

Located 15 km north of Lyon, one of the most important API sites. 1000 people working in a 30 ha space.

Our mission: manufacturing for Healthcare

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Main active ingredients manufactured

Corticostéroïdes

- for Hydrocortisone® and others anti-inflammatory, antiallergic

Dolasétron

- for Anzemet®, antiemetic

Nilutamide

- for Nilandron®, anti-cancer agent

And, in the launching unit

- Dronedarone for Multaq®, Clopidogrel for Plavix®, Eprinomectrine for Eprinex ®

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Introduction

- Most of the time, HPTLC is used for the analysis of Medicinal Plants while HPLC, due to his precision, is mainly used in the pharmaceutical industry. But, in some cases, HPLC has more drawbacks than HPTLC (solvent consumption, equilibration time, matrix effects, need for UV absorbance).

- With four examples, we will present the advantages of using an HPTLC method to monitor chemical reactions during API production.

Example 1: Epoxydation reaction

Analytical objective

- To determine the end of an epoxydation reaction while components have no UV absorbance, the industrial reaction mixture is in CH_2Cl_2 suspension, and the matrix is complex (minerals and organics)

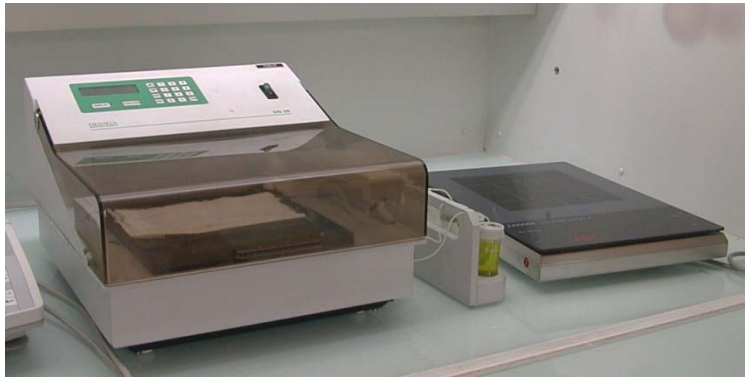
Action expected

- To continue the reaction time if specification is not met

Analytical conditions

- Material: HPTLC plates silica gel 60 F254 (Merck) 20x10 cm
- Application: spay bands of 6 mm, dissolution solvent $CHCl_3$/MeOH : 9/1
- Mobile phase: CH_2Cl_2/MeOH : 97/3
- Development: ADC, front position 50 mm,
  pre-conditioning time 5 mn
- Post-chromatographic derivatization: by spraying phosphomolybdic acid reagent (25g $H_3[PMo_{12}O_{40}].xH_2O$, 500mL CH_3COOH, 25 mL H_2SO_4), heat ~5 mn at 130°C

Example 1: HPTLC apparatus



Total analytical time: 35 mn

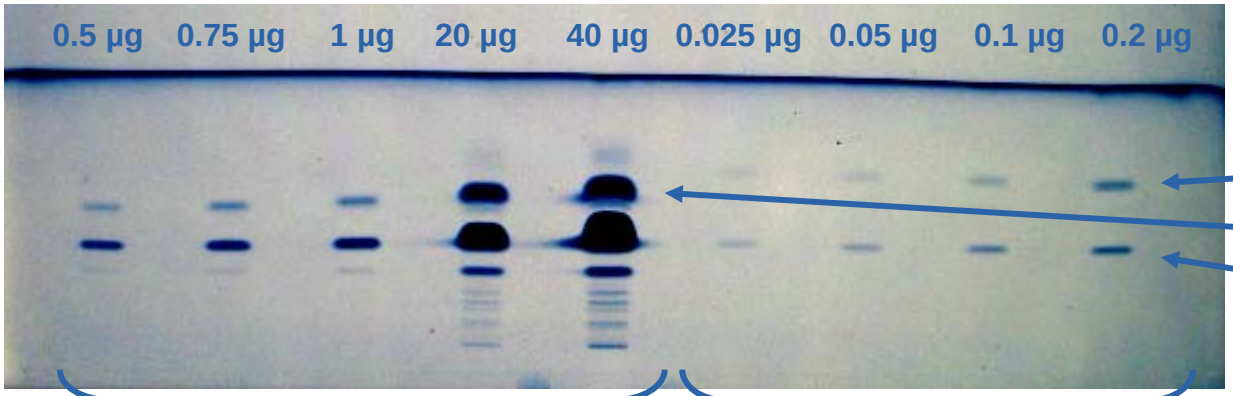
- Solution preparation: 7 mn
- Application: 8 mn
- Pre-conditioning: 5 mn
- Development: 10 mn
- Derivatization: ~5 mn

Apparatus

- Camag ATS 3
- Camag ADC
- Desaga ChromaJet DS20
- Camag TLC Plate Heater III

Example 1: HPTLC results

HPTLC plate after derivatization, under white light



Results

- **Starting material: less than 0.5 %**
- **Beta isomer: between 5 and 10 % (indicative)**

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Example 2: End of washing

Analytical purpose

- Verify elimination of reactant from the main reaction mixture by liquid-liquid extraction

Action expected

- Add more extraction steps if specification not met

Analytical conditions

- Material: HPTLC plates silica gel 60 F254 (Merck) 10x10 cm
- Application: spay bands of 6 mm, dissolution solvent: MeOH+NH_4OH
- Mobile phase: CH_2Cl_2/MeOH/NH_4OH : 47/6/1
- Development: TTC normal, front position 70 mm,
- Post-chromatographic derivatization: by spraying Nihydrin reagent (1g Nihydrin, 50mL EtOH, 10 mL CH_3COOH), heat ~2 mn at 130°C

Example 2: HPTLC apparatus

Total analytical time: 35 mn

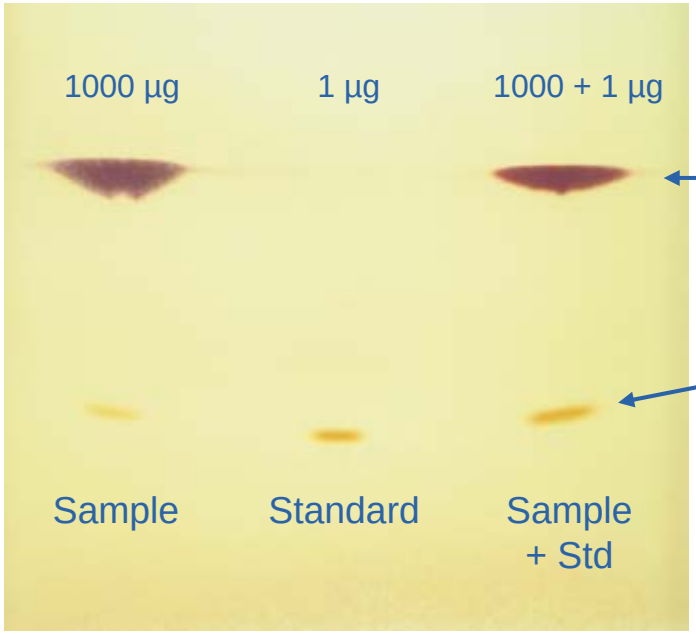
- Solution preparation: 5 mn
- Application: 5 mn
- Development: 20 mn
- Derivatization: ~5 mn

Apparatus

- Camag ATS 4
- Camag TTC and SmartALERT
- Manual spray system
- Camag TLC Plate Heater III

Example 2: HPTLC results

HPTLC plate after derivatization, under white light



Results

- **Impurity: less than 0.1 %**

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Example 3: Reduction reaction

Analytical purpose

- Compare 2 available analytical methods (HPLC and HPTLC)
- Verify that reduction is completed after 3h reaction time

Action expected

- Choose the more reliable method to implement in production routine
- Add reaction time if specification not met

Example 3: Reduction reaction

Analytical conditions for HPTLC

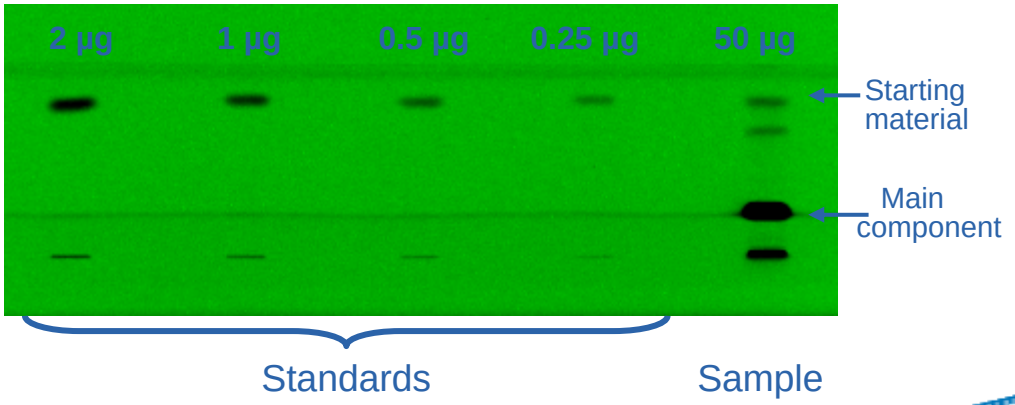
- Material: HPTLC plates silica gel 60 F254 (Merck) 20x10 cm
- Application: spray bands of 6 mm
- Mobile phase: CH_2Cl_2/MeOH : 98/2
- Development: ADC, front position 40 mm
- Examination: UV 254 nm

Analytical time: 20 mn

- Solution preparation: 5 mn
- Application: 5 mn
- Development: 10 mn

Results:

- Impurity ≤ 1%



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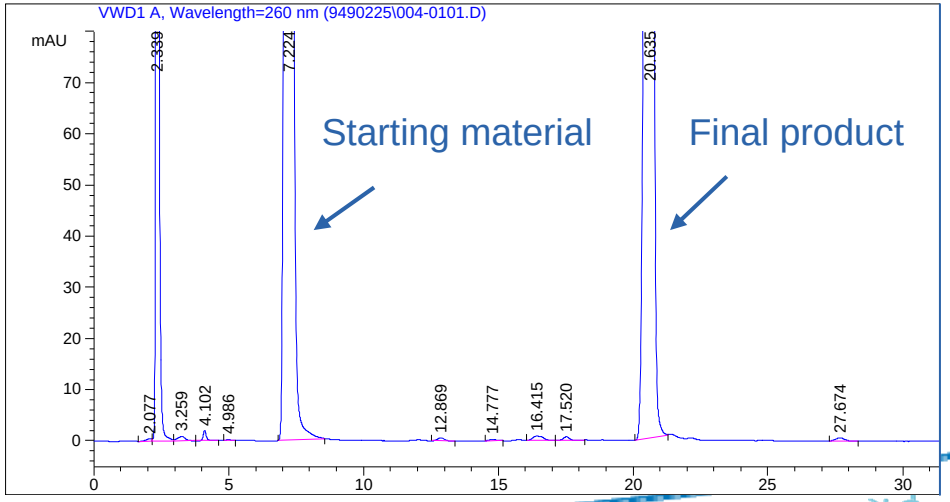
Example 3: Reduction reaction

Analytical conditions for HPLC

- Column: Inertsil C4, 5 µm, 4.6x150 mm
- Mobile Phase A: KH2PO4 (0.02 M)/ACN : 55/45
- Mobile Phase B: KH2PO4 (0.02 M)/ACN : 45/55
- Gradient:

time 0	MPA 100%	MPB 0%
time 10	MPA 100%	MPB 0%
time 16	MPA 0%	MPB 100%
time 40	MPA 0%	MPB 100%

- Flow rate: 1 mL/mn
- UV absorbance: 260 nm
- Temperature: 22°C
- Run time: 30 mn



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Example 3: Reduction reaction

Conclusion

- For routine analysis, to be done by operators during shifts, HPTLC was chosen for its simplicity of implementation:
  - no apparatus for preparation and occupation,
  - no suitability test and standard pre-injection,
  - low solvent consumption,
  - low risk of instrumentation failure,
  - satisfactory precision,
  - analysis time equivalent.

Example 4: End of hydrolysis reaction

Analytical purpose

- Verify if hydrolyze is completed (less than 1 % initial product)
- The analytical method must be as simple as possible in order to minimize investment, analytical time, and implementation.

Action expected

- Add reaction time or acid if specification isn't met

Analytical conditions

- Material: HPTLC pre-scored plates silica gel 60 F254 (Merck) 5x5 cm
- Application: capillary contact
- Mobile phase: CH_2Cl_2/Isopropyl ether/AcOEt : 42/2/6
- Development: TTC 10x5 cm, front position 35 mm
- Post-chromatographic derivatization: immersion in phosphomolybdic acid reagent diluted 50% in MeOH, heat ~5 mn at ~130°C

Example 4: HPTLC apparatus

Total analytical time: 20 mn

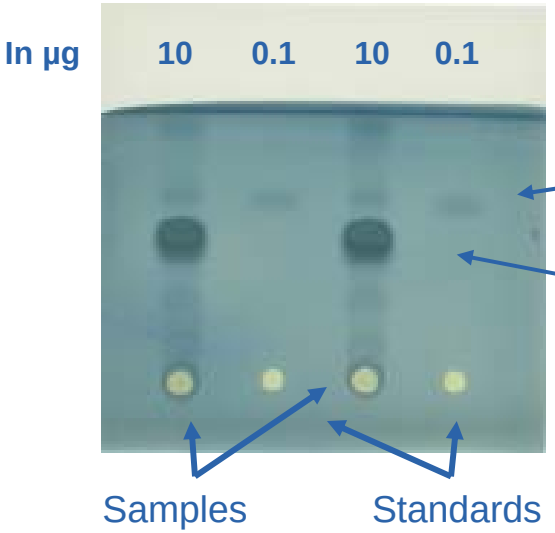
- Solutions preparation: 7 mn
- Application: 1 mn
- Development: 7 mn
- Derivatization: 5 mn

Apparatus

- Camag Nanomat
- Camag Capillary dispenser
- Camag TTC and SmartALERT
- Laboratory devices !

Example 4: HPTLC results

HPTLC plate



Results

- **Starting material ≤ 1 %**

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Conclusion

For those 4 examples of monitoring chemical reactions, HPTLC was chosen by:

- simplicity of implementation by plant operators,
- low risk of instrumentation failure and low cost,
- no needs of instrument conditioning and rinsing,
- possibility of derivatization,
- no problem of matrix effect and column contamination

The methods have to be adapted according to the analytical purpose:

- When precision is required: a complete calibration is necessary,
- When a short analytical time is required: small plates and migration distance,

HPTLC is always possible with or without sophisticated instrumentation