

TLC and HPTLC MS-grade plates for mass spectrometry



TLC and HPTLC MS-grade plates for mass spectrometry coupling

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Simple thin layer chromatography (TLC) is the most widely used technique in planar chromatography, whereas high performance TLC (HPTLC) is considered to be the most efficient and powerful technique. In HPTLC, the silica used has a smaller particle size (4–8 μm) and a narrower particle distribution.

In 1969, Prof. R.E. Kaiser reported the coupling of thin-layer chromatography with mass spectrometry (MS) for the first time. TLC spots were heated and desorbed into a gas stream in front of the source of a mass spectrometer. Later H.J. Issaq demonstrated the use of multi-dimensional TLC coupled to MS, where separated zones were eluted from the TLC plate with methanol and introduced into the MS using the Eluchrom interface (CAMAG special products). Since then, many TLC-MS publications have been made, in particular in the last 3–4 years as interest has strongly grown.

Today, coupling TLC plates to Mass Spectrometry is a new field of high interest, which will contribute strongly to the progress of planar chromatography, today and in the future.

The techniques

The techniques for coupling TLC with mass spectrometry can be divided into:

A

With elution-based techniques, the analyte on the silica plate is dissolved in a solvent and transferred to the mass spectrometer in the liquid phase (see CAMAG interface).

Elution-based techniques

Both approaches are offline, and both are performed after the separation is finished and the plate is dried. The sample transfer to the MS is fast and typically takes less than one minute.

B

With desorption-based techniques, the analyte is vaporized from the silica and transferred to the MS in the gas phase. Vaporization techniques include gas beam, ion bombardment and MALDI (matrix assisted laser desorption / ionisation) or DART (direct analysis in real time).

Desorption-based techniques

Key benefits of TLC-MS are:

- Mass spectra are obtained quickly by direct sample access on the TLC plate at room temperature – high quality spectra are obtained with low background signal.
- Targeted recording of mass spectra on zones or lines of interest is performed after the TLC chromatogram has been developed, thus providing high efficiency.
- One particular advantage of TLC-MS and HPTLC-MS is the flexibility in choosing mobile phases for a separation. By contrast, with standard LC-MS coupling using HPLC, some mobile phases cannot be used (e.g. inorganic buffers).

High efficiency / high resolution

- TLC-MS and HPTLC-MS plates have less impurity in silica gel matrix compared to standard products
- The selectivity of MS-grade plates is the same as Merck Millipore standard TLC and HPTLC plates
- The detection limit of HPTLC-MS-grade plates is in the lower nanogram range

Compatible with different TLC-MS techniques

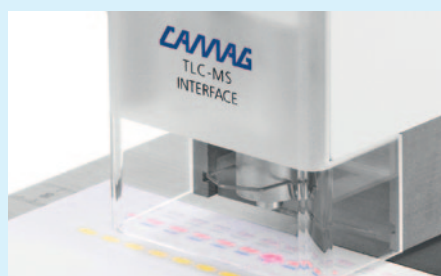
- Elution-based techniques
- Desorption-based techniques

Separation performance

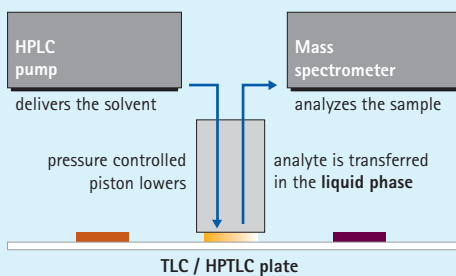
- The separation performance of the new products is equivalent to the standard TLC/HPTLC plates, so that the method with standard TLC plates can be directly transferred to MS-grade plates.

Cleanness

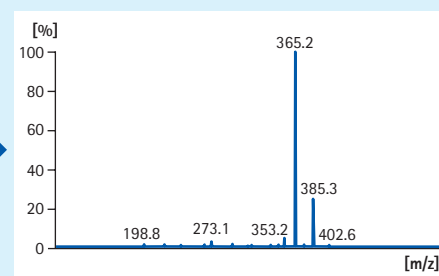
- The important difference between MS-grade plates and standard Merck Millipore plates is that the new MS-grade products are much cleaner (more sensitive, reduced background signals).
- TLC/HPTLC MS-grade plates are packed in aluminum foil to maintain cleanness and prevent contamination.



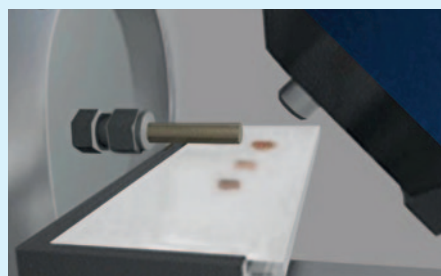
CAMAG interface



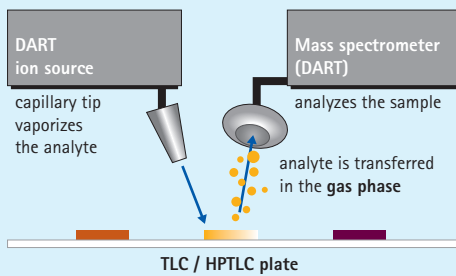
Schema



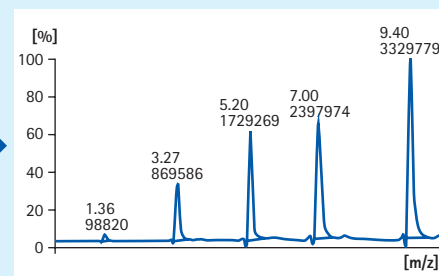
Result



HPTLC-DART-MS interface



Schema



Result

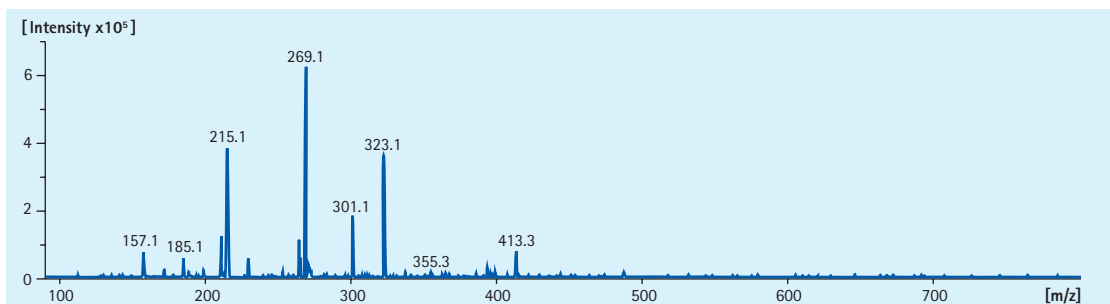
Comparison of HPTLC MS-grade glass plates with Merck Millipore standard HPTLC glass plates under same chromatographic conditions

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The following experimental results demonstrate the enhanced sensitivity of TLC-MS-grade plates:

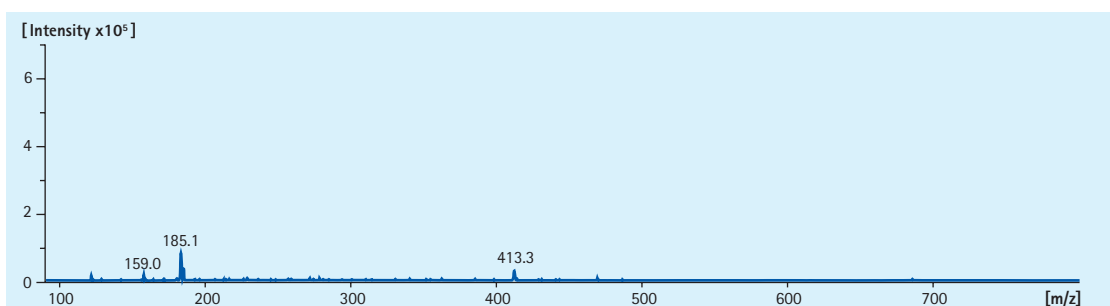
MS background signal measurement using a standard HPTLC silica gel 60 F₂₅₄ glass plate (Ord. No. 1.05642.0001) with mobile phase acetonitrile/water (95/5).

Figure 1



MS background signal measurement using an **MS-grade** HPTLC silica gel 60 F₂₅₄ glass plate (Ord. No. 1.00934.0001) with mobile phase acetonitrile/water (95/5).

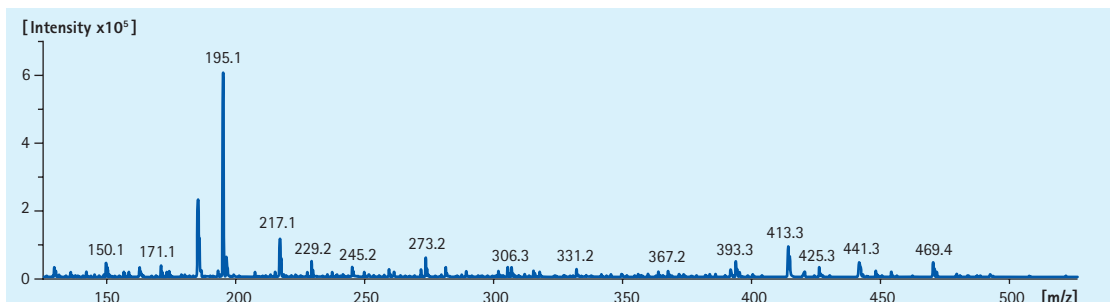
Figure 2



This clearly demonstrates that MS-grade plates have very low background signal compared to standard HPTLC plates.

Trace measurement of caffeine [sample: 20 ng caffeine (MH⁺) 195.1] on a HPTLC silica gel 60 F₂₅₄ **MS-grade** glass plate (Ord. No. 1.00934.0001) with mobile phase acetonitrile/water (95/5) + 0.1% formic acid

Figure 3



ESI-MS mass spectrum of caffeine, measured from a 20 nanogram TLC spot

These experiments clearly demonstrate the performance of the NEW Merck Millipore MS-grade HPTLC plates.

HPTLC Silica gel 60 F₂₅₄ MS-grade glass plates

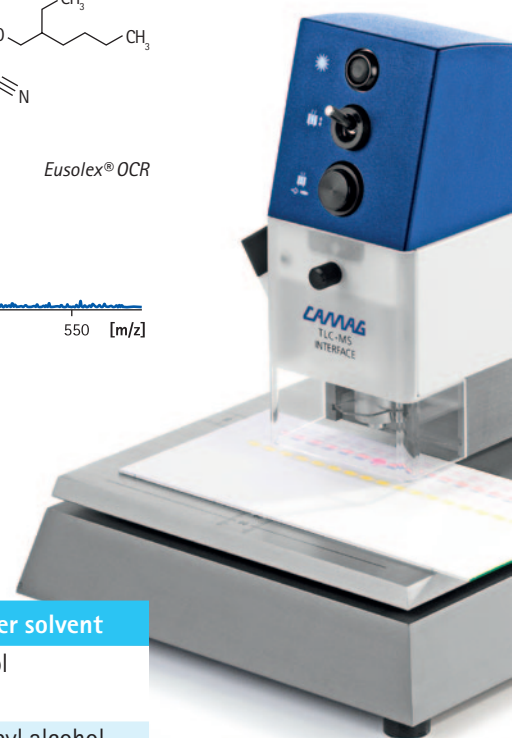
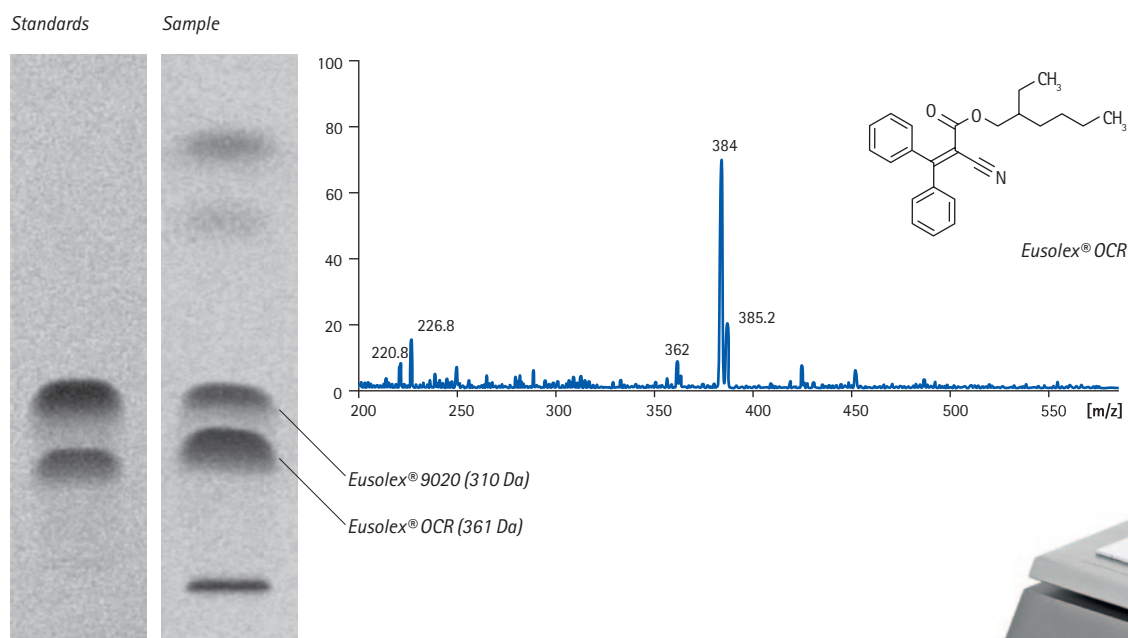
Separation of UV filter in sun cream with spot identification by mass spectrometry

Application 1

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Chromatographic Conditions

Plate	HPTLC Silica gel 60 F ₂₅₄ MS-grade glass plate (Ord. No. 1.00934.0001)
Sample preparation	1 g sun cream in 10 ml isopropyl alcohol at room temperature for 15 min and filtration through a 0.45 µm syringe filter (PTFE Millipore)
Mobile Phase	toluene / n-heptane (6/4, v/v)
Migration distance	5 cm
Migration time	18 min
Chamber	normal chamber without chamber saturation
Detection	Detection UV @ 254 nm
Identification	TLC-MS Interface CAMAG/ESI (+) mode (electrospray ionization)



No.	Volume	Compounds	Sample	Concentration	Transfer solvent
1	1.0 µl	Eusolex® 9020 Eusolex® OCR	standard	1 mg/ml	ethanol
2	1.0 µl	Eusolex® 9020 Eusolex® OCR	suncream	–	isopropyl alcohol

HPTLC Silica gel 60 F₂₅₄ MS-grade glass plates

Separation of steroids with peak identification by mass spectrometry

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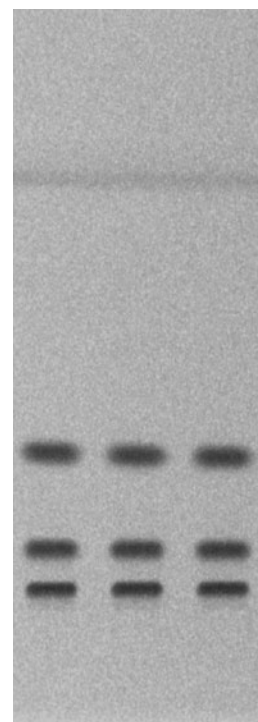
Application 2

Chromatographic Conditions	
Plate	HPTLC Silica gel 60 F ₂₅₄ MS-grade glass plate (Ord. No. 1.00934.0001)
Mobile Phase	petroleum benzene / acetone (8/2, v/v)
Migration distance	5 cm
Migration time	15 min
Chamber	normal chamber without chamber saturation
Staining	no
Detection	Detection UV @ 254 nm
Identification	TLC-MS Interface CAMAG/ESI (+) mode

Methyltestosterone (302 Da)

Reichstein' S (346 Da)

Hydrocortisone (362 Da)

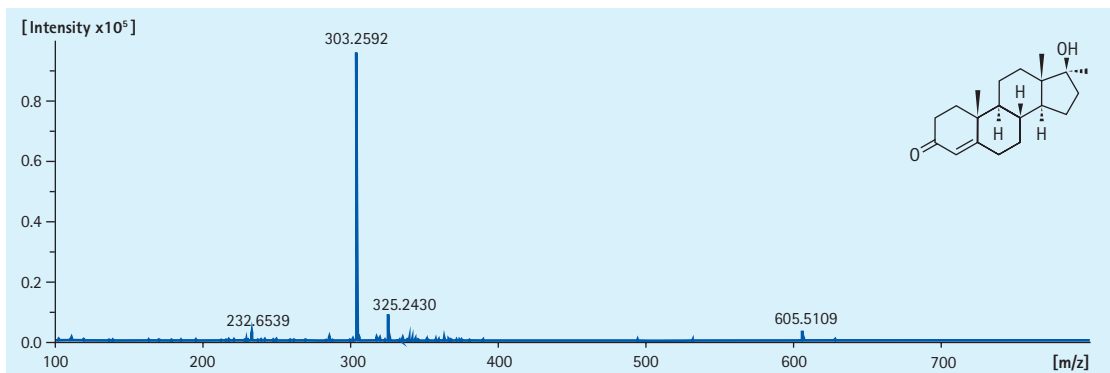


Application result

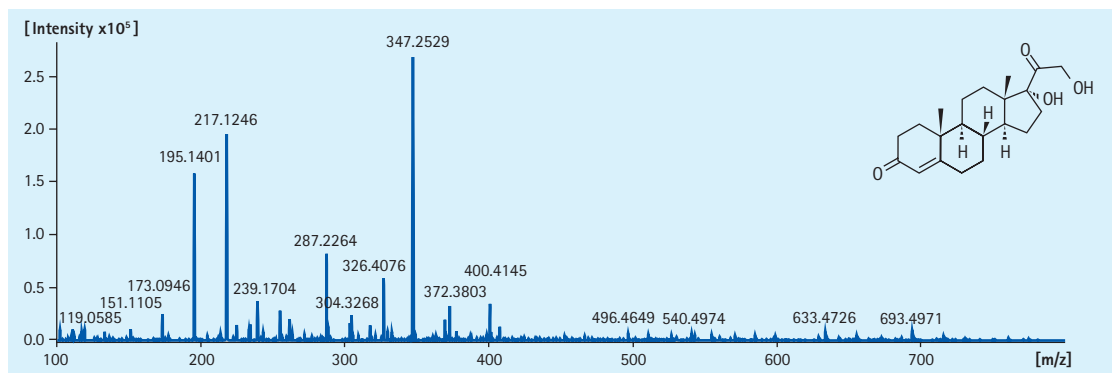
No.	Volume	Compounds	Sample	Concentration	Transfer solvent
1 to 3	2.0 µl	Hydrocortisone	steroids mixture	1.2 mg/ml	methanol
		Reichstein' S		1.0 mg/ml	
		Methyltestosterone		0.8 mg/ml	

See corresponding mass spectrums of Methyltestosterone (figure 5), Reichstein' S (figure 6) and Hydrocortisone (figure 7).

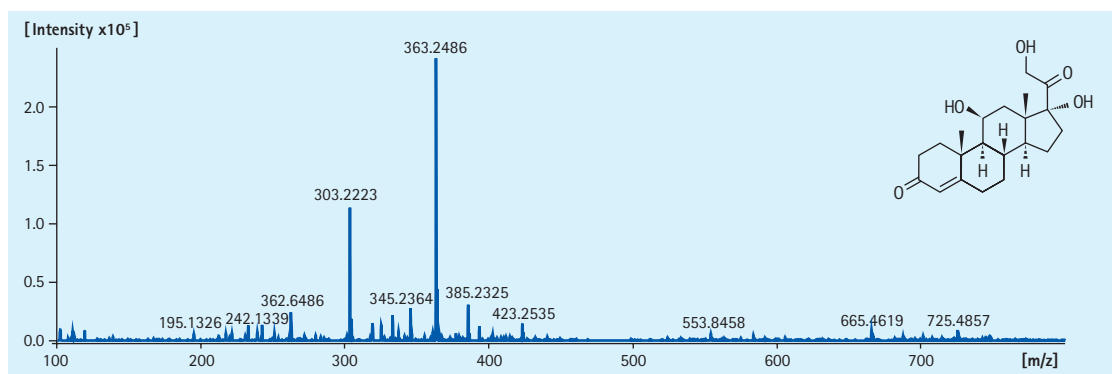
Figure 5



Mass spectrum of Methyltestosterone (TLC-MS Interface CAMAG / ESI (+) mode)



Mass spectrum of Reichstein's (TLC-MS Interface CAMAG / ESI (+) mode)



Mass spectrum of Hydrocortisone (TLC-MS Interface CAMAG / ESI (+) mode)

Summary

The separation efficiency and selectivity of the new MS-grade plates is equivalent to the standard TLC/HPTLC plates; the only difference is that the new products are much cleaner than the standard plates. This gives higher sensitivity and reduced background signals, allowing trace analysis with mass spectrometry detection in the lower nanogram range (see figure 3).

Ordering information

Product name	Comments	Ord. No.
TLC silica gel 60 F ₂₅₄ MS-grade 25 glass plates 20x20 cm	Elution- and desorption-based approach	1.00933.0001
HPTLC silica gel 60 F ₂₅₄ MS-grade 25 glass plates 20x10 cm	Elution- and desorption-based approach	1.00934.0001
HPTLC silica gel 60 RP18 F _{254s} MS-grade 25 glass plates 20x10 cm	Elution- and desorption-based approach	1.51161.0001
HPTLC silica gel 60 F ₂₅₄ MS-grade for MALDI* 20 aluminum foils 5x7.5 cm	Elution- and desorption-based approach	1.51160.0001

*only aluminum plates are suitable for MALDI

Figure 6

Figure 7



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