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Analytical Products

Application Note

Reaction Monitoring of the Esterification Progress of Benzoic Acid by using Thin-Layer Chromatography (TLC) followed by a Substance Identification with Mass Spectrometry (MS)

Safe and secure UV handling using TLC Explorer Documentation System

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Introduction

Thin-layer chromatography (TLC) is an easy, cost-effective, and flexible technique for quick chromatographic analysis, and it is therefore often used in organic synthesis laboratories. Rapid analysis of starting materials, evaluation of purity for starting and reaction product, as well as monitoring the progress of a reaction, are typical application examples using TLC.

In this study, a qualitative reaction progress was analyzed. The formation of methyl benzoate by esterification of benzoic acid with methanol was chosen as model reaction (**Figure 1**). Methyl benzoate has a strong characteristic smell, and it is used in perfumery. It can also be utilized as starting material for transesterification to benzoate plasticizers.[1]

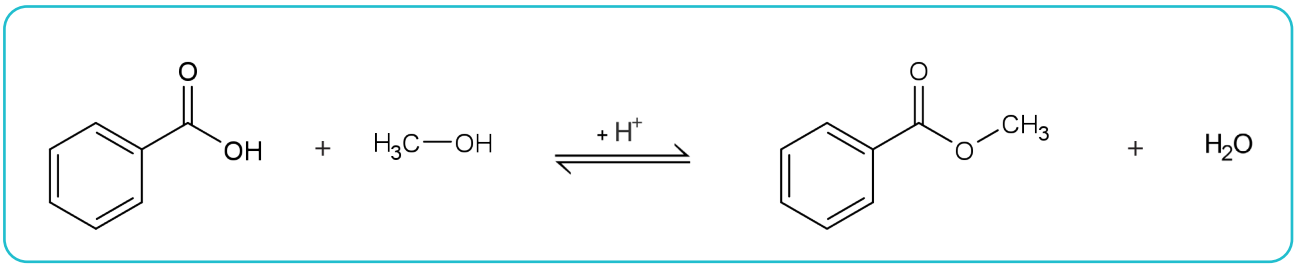

Figure 1. Reaction mechanism of esterification of benzoic acid to methyl benzoate.

Small aliquots of starting material as well as successive aliquots of the reaction mixture during the reaction were spotted on the TLC plate by microcapillary followed by a chromatographic separation. A mixture of toluene/ethanol was chosen as the mobile phase. After TLC development, images of the plates were recorded in parallel using TLC Explorer Documentation System (**Figure 2**, plate 1-4).

A final substance identification was done by TLC-MS transferring an eluted spot of benzoic acid and the purified product methyl benzoate to MS utilizing a TLC-MS interface. This enabled the dissolution of the analyte from the silica plate by a solvent and a transfer to the mass spectrometer in the liquid phase.[2]

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The TLC Explorer Documentation System is a superior instrumental and software solution for reliable TLC plate analysis. It allows the analysis of one or multiple plates in parallel and it is equipped with an integrated safety design ensuring a safe handling with no exposure of UV light. Typically, reaction monitoring in organic laboratories are often done by simple UV lamps risking a direct exposure to UV light that may be hazardous to the unprotected eye. If required, a quantitative evaluation of chromatographic plates is done based on video densitometry. By analysis of obtained chromatograms as a function of reaction time, it is also possible to calculate the conversion percentage of the reactions. The TLC Explorer Documentation system allows a reliable quantification as demonstrated in other applications notes.

Table 1. Chromatographic conditions of synthesis control of methyl benzoate.

Conditions		
TLC	**Plate:**	TLC silica gel 60 F254 MS-grade 5x7.5 cm
	Plate:	1. Start of the reaction 2. Reaction after 1 hour 3. Reaction after 2 hours 4. Purified product
	Mobile phase :	Toluene / Ethanol 9:1 (v/v)
	Migration distance:	5 cm
	Development time:	8 Minutes
Detection	**Documentation:**	TLC Explorer
	Wavelength:	UV light 254 nm
	hRf-Value:	Benzoic acid - 40 Methyl benzoate – 85
	MS-Measurement:	TLC-MS Interface from Camag®, LTQ XL from Thermo Fisher
	Solvent:	Acetonitrile / Water 95:5 + 0.1% acetic acid
	Measured mass:	Benzoic acid 120.8 m/z (M-H) Methyl benzoate – 136.9 m/z (M-H)

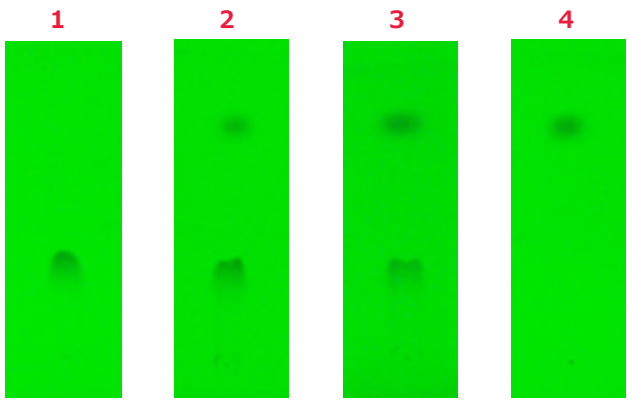

Figure 2. TLC chromatogram analysis of plate 1 – 4 by the TLC Explorer under UV 254 nm excitation. Plate 1: Starting mixture before reaction has started. Plate 2: Reaction mixture after 1 hour duration. Plate 3. Reaction mixture after 2 hours. Plate 4. Purified product.

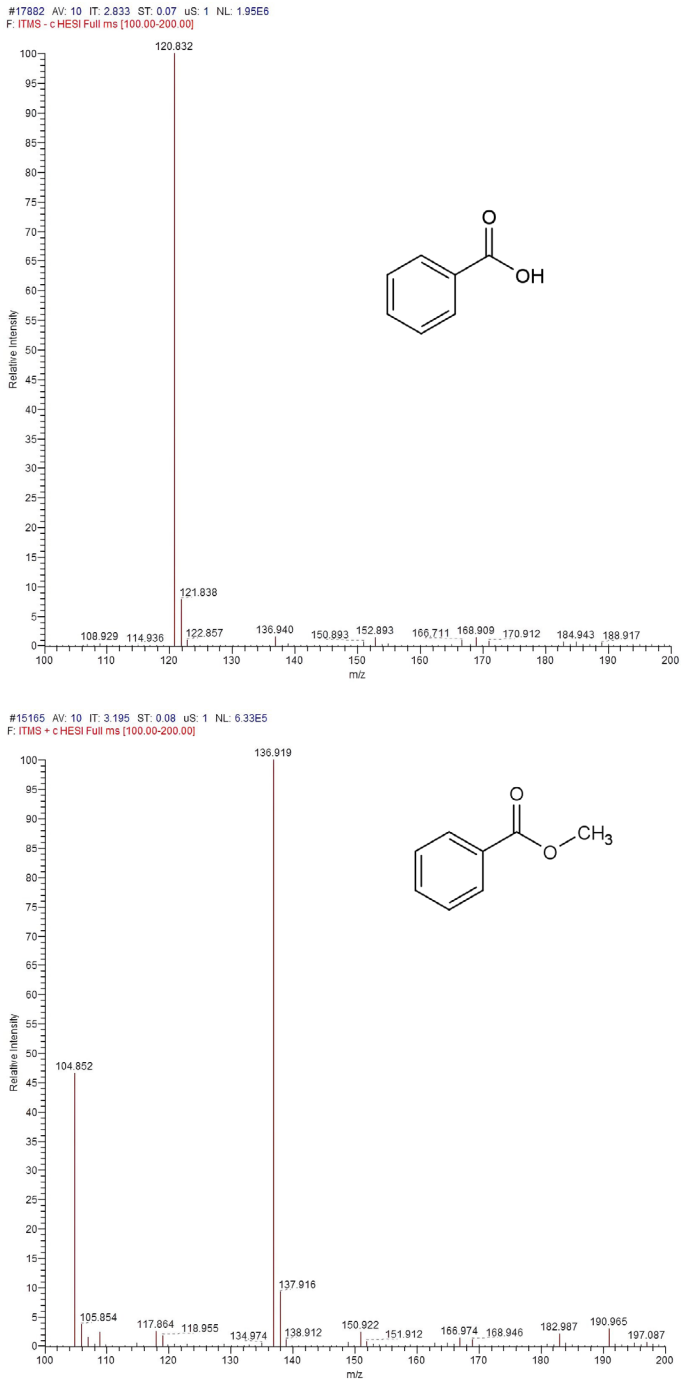

Figure 3. MS spectrum of starting compound benzoic acid (top) and MS spectrum of purified reaction product methyl benzoate (bottom).

Discussion

As demonstrated, the use of the TLC Explorer allowed a reliable and convenient data taking of several plates in parallel including automated Rf recognizing of the spots. A straightforward analysis of the reaction progress was easily possible including a simple download of the image. Besides, a safe handling was ensured due to it's safe design concept. A direct exposure of UV light is prevented.

Featured Products

Description	Cat. No.
TLC Explorer	**1.52610**
TLC silica gel 60 F254 MS-grade, 5x7.5 cm plates	**1.51022**
Toluene for liquid chromatography LiChrosolv®	**1.08327**
Ethanol for liquid chromatography LiChrosolv®	**1.08327**
Methyl benzoate analytical standard	**18344**
Benzoic acid ACS reagent ≥99.5%	**242381**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Water for chromatography (LC-MS Grade) LiChrosolv®	**1.15333**
Acetic acid for LC-MS LiChropur™	**5.33001**

Reference

1. Maki, Takao; Takeda, Kazuo. "Benzoic Acid and Derivatives". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley.
2. Schulz M., Oberle M., Burholt M., Baeumle M. 50 years of TLC-MS Thin-Layer Chromatography coupled to Mass Spectrometry and new perspectives by complementary use to HPLC as demonstrated in testing of honey. Analytix Reporter. 2019;5: 3-7.SigmaAldrich.com/analytix
3. Unpublished data

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03/2024