REAL-TIME REACTION MONITORING OF A SOLUTION-PHASE PEPTIDE SYNTHESIS USING THE PLATE EXPRESS™ AND expression CMS

APPLICATION NOTE

Mass Spec: expression CMS
Sampling: Plate Express™

Advion, Inc.

In this application note, TLC/CMS with the Advion Plate Express TLC Plate Reader and expression Compact Mass Spectrometer (CMS) is used for real-time reaction monitoring of peptides of pharmaceutical interest to overcome synthetic challenges and optimize chemical reactions directly in the lab.

INTRODUCTION

Real-time reaction monitoring based on a combination of thin-layer chromatography (TLC) and compact mass spectrometry (CMS) is a simple and quick way for chemists to overcome synthetic challenges and optimize chemical reactions in the modern laboratory. Peptides of pharmaceutical interest can be readily synthesized following a rapid, continuous solution-phase synthesis strategy based on Fmoc protected amino acid building blocks. A simple model for such a reaction is the growing of analogues of the acyl carrier protein (ACP), a component of the fatty acid synthesis pathway[1].

METHOD

Reaction Procedure

Fmoc-Asn-(Trt)-OH (1.0 mM, 600 mg) and O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU, 1.0 mM, 400 mg) were dissolved in 10 mL dichloromethane (DCM) in a 50 mL round-bottom flask and stirred. The amino acid building block was activated by adding *N,N*-Diisopropylethylamine (DIEA, 2.65 mM, 465 µL) and stirred for 5 min. The Glycine *tert*-butyl ester hydrochloride (H-Gly-OtBu, 0.65 mM, 109 mg) was added and the reaction commenced for 1 hr at room temperature.

The reaction mixture was diluted with 10 mL DCM and washed with a series of 10 mL each of 10 vol% monosodiumcarbonate, water, and brine. The DCM was dried over magnesium sulfate, filtered, and the product was de-blocked by the addition of tris(2-aminoethyl)amine (TAEA, 3.5 mL). This mixture was stirred for 5 min and washed with 7 mL each of brine, phosphate buffer (1.76 mol/L, pH to 5.5) and brine again. The clear organic layer contained the dipeptide product of step one.

The reaction can continue until the final amino acid length is reached.

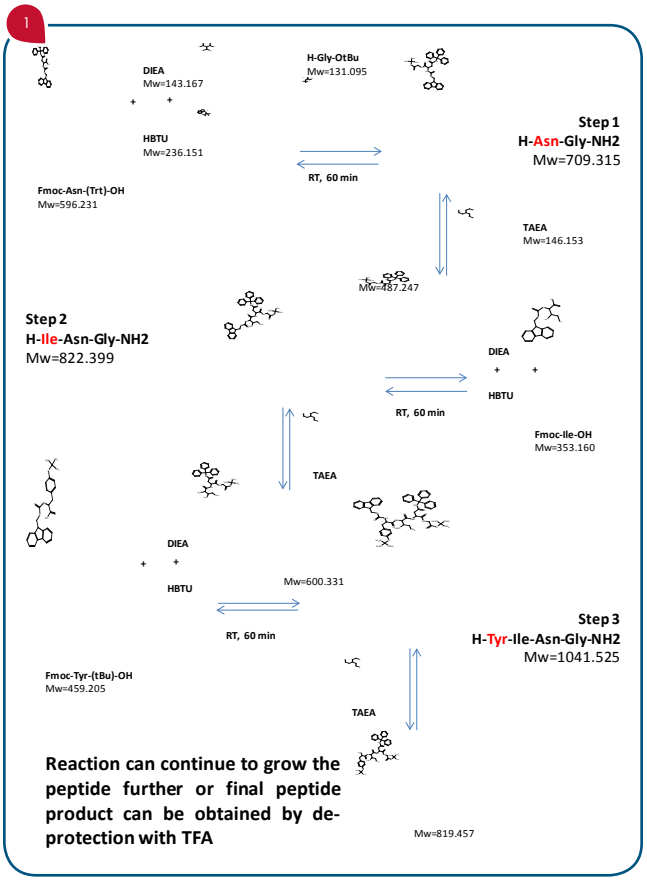

Figure 1: Reaction scheme for the continuous growth of a peptide of pharmaceutical interest following the Fmoc building block strategy in homogeneous phase as described by Carpino et al.[1].

Sample Collection and Development of TLC Plates

1 µL of reaction mixture was taken at different time points of the 1 hour reaction and placed onto the origin of a TLC plate (Merck EMD, 5534-3 Silica Gel 60 F254, 5 x 20 cm, 0.2 mm). The TLC plate was developed using a mixture of DCM/Methanol/Acetic acid (9:1:0.1). TLC spots were located under UV light exposure at 254 nm.

TLC/CMS Analysis

TLC spots were further processed with the Advion Plate Express TLC Plate Reader. The TLC plate was moved underneath the extraction head and the target area illuminated with a laser spot. The extraction spot area had an oval shape of 2 x 4 mm. The contact pressure was selected at 280 Nm and a solvent flow of 200 µL/min 80/20 ACN/water 0.1 vol% formic acid delivered the extracted analytes to the Advion expression CMS via electrospray ionization. The CMS was set to scan from *m/z* 100 to *m/z* 1200 for the 30 second acquisition time of the TLC/CMS experiment.

Figure 2: Instrument setup with the CMS and Plate Express for TLC/CMS.

RESULTS

TLC/CMS Enables Direct Reaction Monitoring

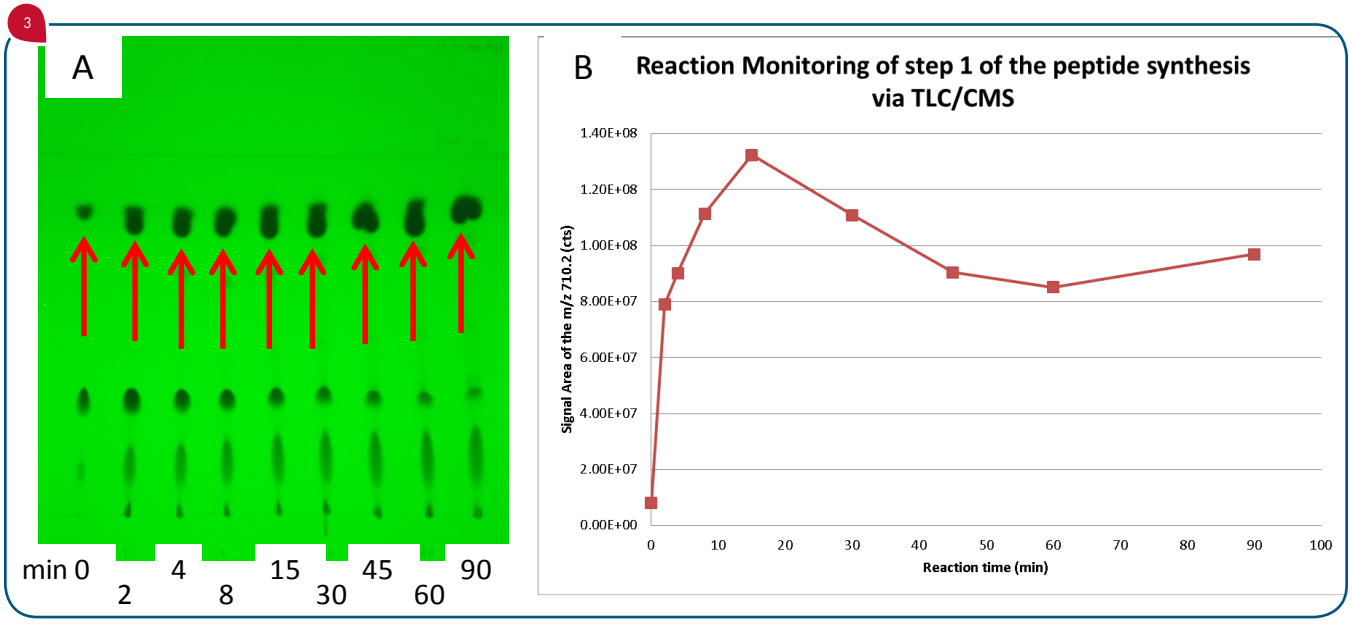

Figure 3: (A) TLC Plate and (B) monitoring of the first reaction step of the peptide synthesis.

The first reaction step of the peptide synthesis was monitored by placing 1 µL of the reaction mixture on a silica TLC plate and developing the plate at the end of the reaction time. Fluorescent light showed a multitude of compounds being separated on the TLC plate (Figure 3A). Subsequent TLC/CMS analysis of the spots along the 15 min lane (data not shown, see Figure 5 for example) showed that Spot 4 represented the desired product (red arrows). The blocked product was detected at *m/z* 732.20, as the $(M+Na)^+$ signal. Detected signal area intensity of the respective spots show an intensity-time curve consistent with a completed reaction at ~20 min with a following loss of product in contrast to the literature suggested reaction time of 60 min.

Applicable for a Broad Range of Chemical Compounds Including Protected and Unprotected Peptides

TLC separation after the clean up of the reaction solvent showing both the fmoc blocked (left) as well as unblocked (right) dipeptide product from reaction Step 1. The mass spectra of both products at their respective $(M+Na)^+$ ions can be seen at *m/z* 732.21 and *m/z* 510.15 (Figure 4).

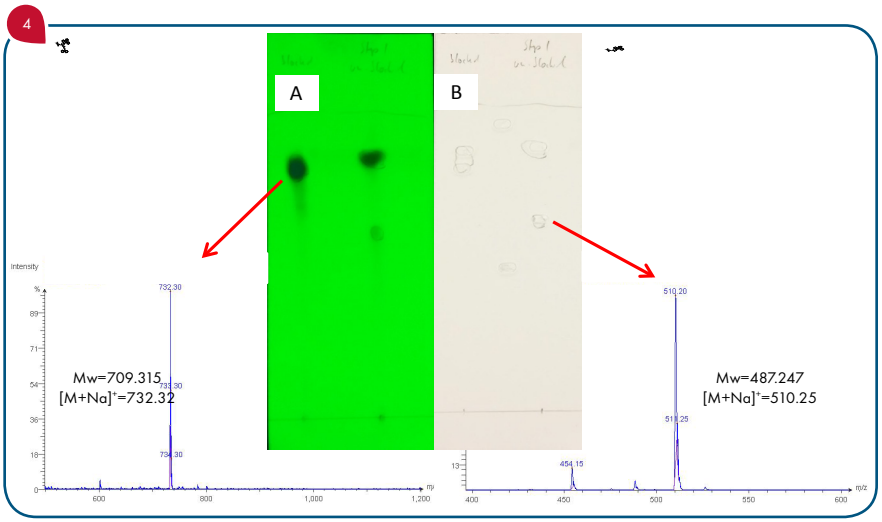

Figure 4: (A) Fluorescence response and (B) mass spectra of TLC plate after extraction.

Identification of Reactants and Products of Chemical Reactions Directly via TLC/CMS

TLC/CMS can identify the product spot of interest in a complex mixture separated on silica gel TLC plate. Spot 2 showed the expected *m/z* signal of the reactant (lower trace) and Spot 6 showed the *m/z* signal of the desired Fmoc blocked four amino acid peptide (Figure 5). Both analytes were detected as the $(M+Na)^+$ ion.

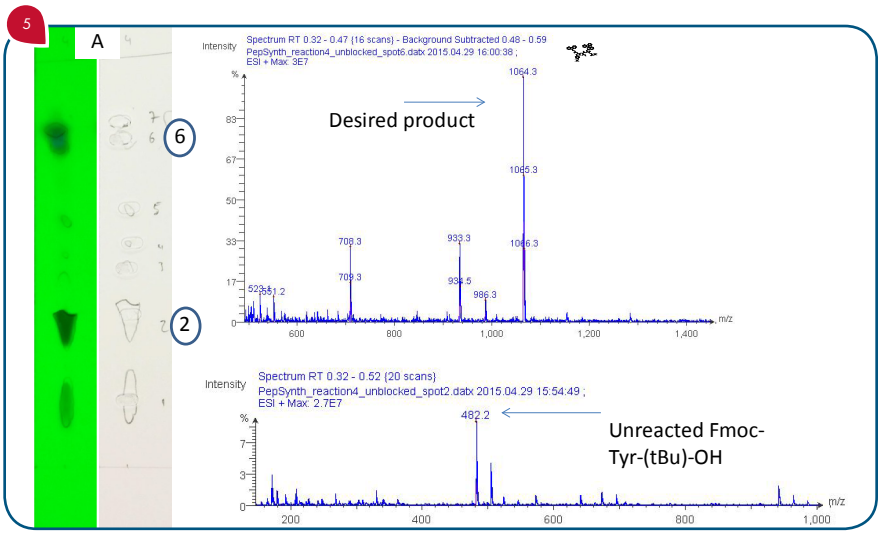

Figure 5: (A) Developed TLC plate under UV light (left) and after extraction (right) and (B) mass spectra of Spot 2 and 6 of the reaction.

Reaction Process Control Across Many Steps of a Continuous Solution Phase Peptides Synthesis Reaction

Following the growing Fmoc protected peptide with TLC/FIA/MS analysis to confirm the correct product is build during the initial three steps of the reaction sequence.

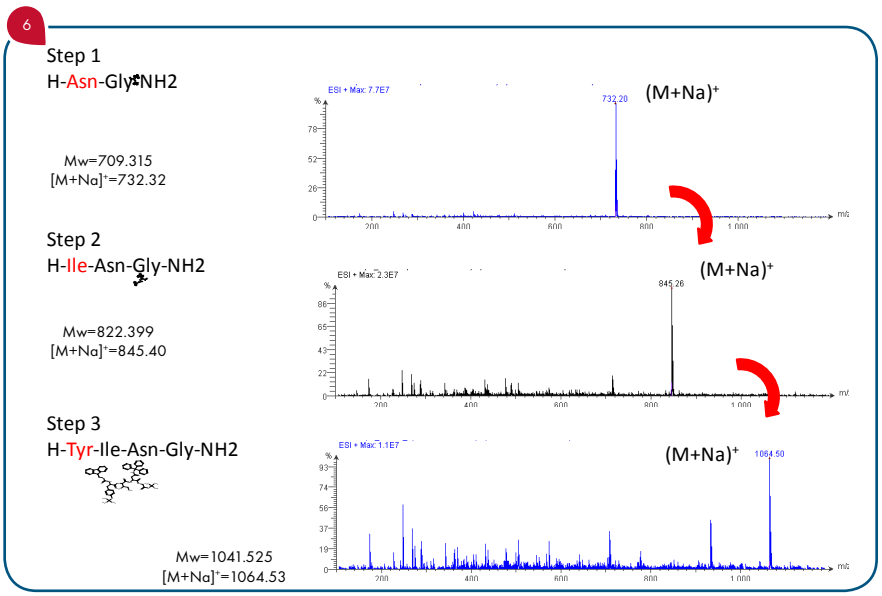

Figure 6: Mass spectra of each step of the reaction sequence.

CONCLUSIONS

- TLC/CMS analysis using the Advion expression CMS and Advion Plate Express TLC Plate Reader can identify reactants, products and side products of a chemical peptide synthesis reaction.
- Both Fmoc blocked and unblocked peptides, as well as the final four amino acid peptide could be detected.
- TLC/CMS can monitor the chemical reaction of the peptide synthesis and showed that the reaction was indeed complete after only 15 min, rather than the expected and literature reported 60 min.
- TLC/CMS is an ideal combination of flexible and easy sample preparation with detailed and powerful analyte detection for synthetic chemical reaction monitoring.

LITERATURE & ACKNOWLEDGEMENTS

[1]Carpino LA, Ghassemi S, Ionescu D, Ismail M, Sadat-Aalaee D, Truran GA, Mansour EME, Siwruk GA, Eynon JS and Morgan B: Rapid, continuous solution-phase peptide synthesis: Application to Peptides of pharmaceutical interest. Organic Process Research and Development 2003, 1(7), 28-37.

We would like to thank Shahnaz Ghassemi from Synpure LLC for technical assistance in the peptide synthesis.