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Advion is a leader in mass spectrometry & synthesis solutions. The expression CMS is a high performance, compact and affordable single quadrupole mass spectrometer. Its compact size allows it to fit in space-limited laboratories for direct access and immediate results for chemists requiring mass confirmation, reaction monitoring, quality control and purity analysis.

Application Note

Real Time Reaction Monitoring of a Solution Phase Peptide Synthesis
Using TLC/Compact Mass Spectrometry

Introduction

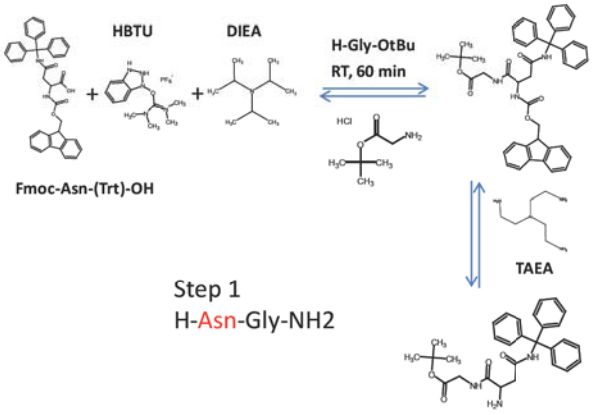
Real-time reaction monitoring based on the expression compact mass spectrometer (CMS) can support the synthetic chemist to overcome synthesis challenges and optimize the reaction time on-line. Peptides of pharmaceutical interest can be readily synthesized following a rapid, continuous solution-phase synthesis strategy based on Fmoc protected amino acid building blocks[1]. 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. Thin layer chromatography (TLC) is a simple and easy way to prepare complex reaction samples for CMS analysis following a TLC/CMS approach.

Reaction Procedure

Fmoc-Asn-(Trt)-OH (1.0 mmol, 600 mg) and O-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 1.0 mmol, 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 mmol, 465 µL) and stirred for 5 min. The Glycine *tert*-butyl ester hydrochloride (H-Gly-OtBu, 0.65 mmol, 109 mg) was added and the reaction commenced for 1 h at room temperature.

The Fmoc protected dipeptide can be unblocked using tris(2-aminoethyl)amine.

Reaction Pathway



The reaction can be continued for more additions of amino acid building blocks (not shown in the reaction pathway), before the final de-protection of the product with trifluoroacetic acid (TFA).

Development of TLC plates

TLC plates (Merck EMD, 5534-3 Silica Gel 60 F254, 5x20 cm, 0.2 mm) were developed using a mixture of Dichloromethane/Methanol/Acetic acid (9:1:0.1). TLC plates were monitored by a hand held UV light excitation at 254 nm.

TLC spot extraction

Developed TLC plates were further processed with a semi-automated TLC spot extractor 'TLC/MS Interface' (Camag Scientific, Wilmington, NC) for TLC/CMS analysis. The TLC card was moved underneath the interface and the target area illuminated with a laser cross. The extraction head had an oval extraction shape of ca. 3x5 mm and an extraction area of ca. 15 mm^2. The head was pressed against the TLC plate using 25 psi pressure and a flow of 200 µL/min 80/20 Acetonitrile/water 0.1 vol% formic acid was flushed through the extraction head and directly to the ESI/MS source.

MS method settings

The expression Compact Mass Spectrometer (CMS) (Advion Inc., Ithaca, NY) was set to scan from *m/z* 100 to *m/z* 1200 for the 3 min acquisition time in the TLC/CMS experiments.

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Results

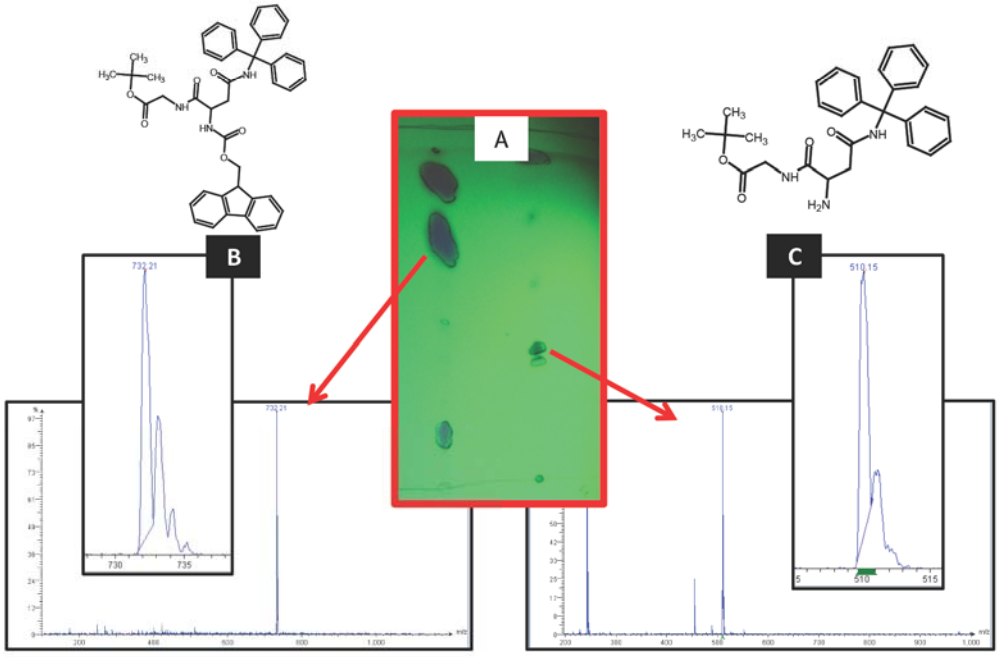

Figure 1: TLC separation after clean up of the reaction solvent showing both the Fmoc blocked (left) as well as un-blocked (right) dipeptide product from reaction Step 1 (A). TLC/CMS analysis can confirm both product forms via their respective $(M+Na)^+$ ions at *m/z* 732.21 (B) and *m/z* 510.15 (C).

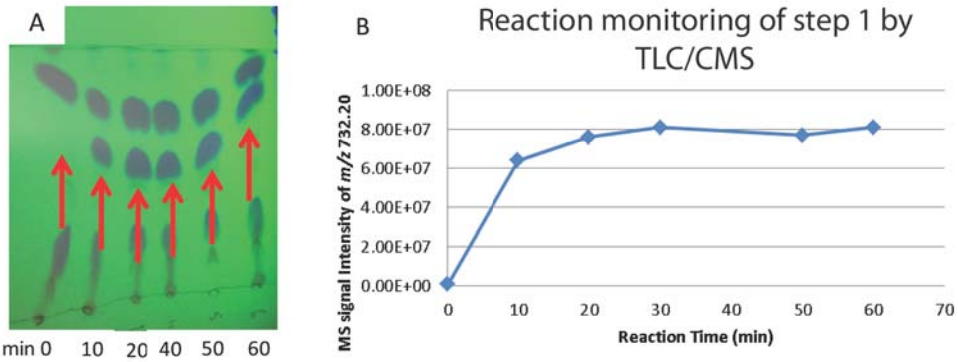

Figure 2: Monitoring the first reaction step every 10 min by placing 1 µL of the reaction solvent on a silica TLC plate and developing the plate at the end of the reaction time. UV light showed a multitude of compounds being separated on the TLC plate (A). Subsequent TLC/CMS analysis of the spots along the 10 min lane (data not shown) showed that spot 4 represented the desired product (red arrows). The Fmoc blocked product was detected at *m/z* 732.20, the $(M+Na)^+$ signal. Signal intensity of the respective spots show a time curve consistant with a completed reaction at 20 min.

Summary

- Silica gel TLC/CMS was able to identify educts, products and side products of a chemical peptide synthesis reaction
- Both Fmoc blocked as well as unblocked peptides could be detected as the $(M+Na)^+$ ion in the mass spectrometer
- TLC/CMS could monitor the chemical reaction and showed that the reaction was complete in 20 min rather than the expected 60 min.

Literature/Acknowledgement

[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

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