Compact mass spectrometer (CMS) analysis of uniformly ^{13}C-labeled amino acids to identify authentic therapeutic drugs and protect against counterfeits

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Advion

Overview:

Cost effective, multidimensional GRAS (generally recognized as safe) counterfeit protection of drugs based on uniformly ^{13}C labeled amino acids with compact mass spectrometry detection.

Introduction:

- Counterfeit drugs are a significant health risk to consumers and an economic burden to the pharmaceutical industry.
- A novel strategy for the unique serialization of genuine drugs can be achieved by addition of uniformly ^{13}C labelled amino acids (AA) with highly patterned isotopic *m/z* signals [1,2].
- Isotopic ratio outlier analysis (IROA) utilizing compact mass spectrometry (CMS) is a simple and cost effective analysis approach (Figure 1).

Figure 1: LC/CMS set up utilizing an expression^{1} compact mass spectrometer and gradient pump LC system (Advion Inc.) for IROA amino acid analysis from tablet examples.

Methods and Materials:

Uniformly ^{13}C labeled glutamine and cysteine were selected as GRAS compliant amino acids. They were dissolved in ethanol at one mg/mL and Tic Tacs® (Ferrero Inc.) were placed shortly into the mixture, air dried and stored in an Eppendorf tube for further processing (Figure 2). Samples were generated by dissolving the Tic Tac® in 0.1 N HCl at 50 mg/mL. 2-200 µL sample was injected onto an Intrada Amino Acid 100 mm x 3 mm column (Imtakt USA, OR) with the following LC gradient settings: 0-3 min at 5 %B; 3-20 min from 5 to 95 %B; 20-22 min from 95 to 5 %B; 22-25 min at 5 %B, with Solvent A (acetonitrile with 0.1 vol% formic acid) and Solvent B (100 mM ammoniumformate) at a total flow rate of 500 µL/min.

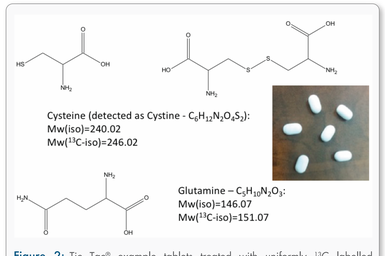

Figure 2: Tic Tac® example tablets treated with uniformly ^{13}C labelled Glutamine and Cysteine.

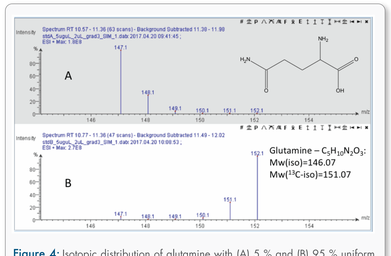

Figure 4: Isotopic distribution of glutamine with (A) 5 % and (B) 95 % uniform ^{13}C label. This specific pattern and relative abundance (IROA) allows for serialization of the drug coating, could be unique to the production batch, and can not be mimicked by other means.

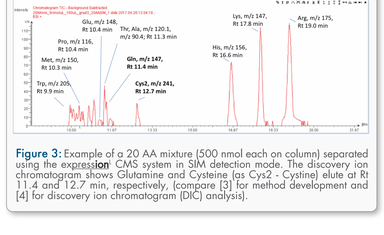

Figure 3: Example of a 20 AA mixture (500 nmol each on column) separated using the expression^{1} CMS system in SIM detection mode. The discovery ion chromatogram shows Glutamine and Cysteine (as Cys2 - Cystine) elute at Rt 11.4 and 12.7 min, respectively, (compare [3] for method development and [4] for discovery ion chromatogram (DIC) analysis).

Under these conditions, the amino acid Glutamine eluted at Rt of 11.4 min and Cysteine eluted and was detected as the oxidation product Cystine at Rt 12.7 min (Figure 3) with characteristic ^{13}C enriched isotopic patterns (example for Glutamine shown in Figure 4). The expression^{1} CMS was operated in scan mode from *m/z* 120 to 250 with a scan time of 300 ms or in SIM mode with *m/z* 147.1, 148.1, 149.1, 150.1, 151.1, 152.1 for Glutamine and 241.1, 242.1, 243.1, 244.1, 245.1, 246.1 and 247.1 for Cysteine (as oxidized Cystine) at dwell times of 50 ms and span of *m/z* 0.3 each.

Results:

- Relative abundance ratios are defined by the uniformity of the label and the mixture ratio of the two batches of e.g. Glutamine and can be used to serialize tablets by various combinations.
- SIM mode shows higher sensitivity of the AA detection and simpler visualization of the isotopic pattern.
- LC/CMS shows the unique isotopic pattern and enriched A+1 signal, suggesting a 5% level enrichment can reliably be distinguished by IROA analysis.
- Example Tic Tac® tablets coated with uniformly ^{13}C labeled Glutamine and/or Cysteine can easily be distinguished from each other by using only two different levels of enrichment.

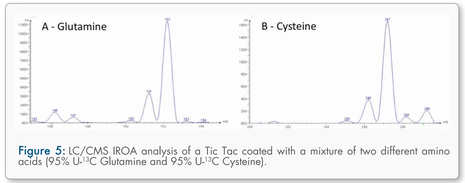

Figure 5: LC/CMS IROA analysis of a Tic Tac coated with a mixture of two different amino acids (95% U-^{13}C Glutamine and 95% U-^{13}C Cysteine).

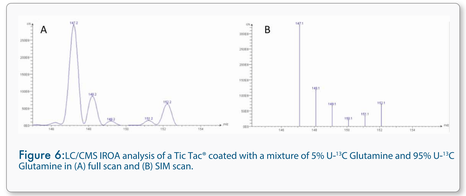

Figure 6: LC/CMS IROA analysis of a Tic Tac® coated with a mixture of 5% U-^{13}C Glutamine and 95% U-^{13}C Glutamine in (A) full scan and (B) SIM scan.

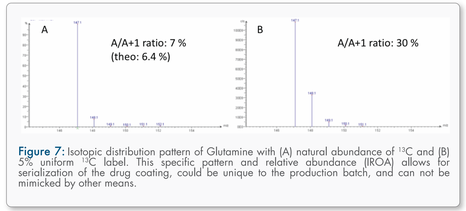

Figure 7: Isotopic distribution pattern of Glutamine with (A) natural abundance of ^{13}C and (B) 5% uniform ^{13}C label. This specific pattern and relative abundance (IROA) allows for serialization of the drug coating, could be unique to the production batch, and can not be mimicked by other means.

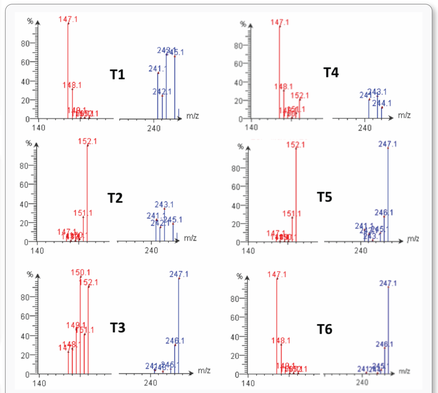

Figure 8: Six Tic Tac® Tablets (T1 to T6) analyzed by LC/CMS for 13C-labeled Glutamine (red, left side) and ^{13}C- labeled Cysteine (blue, right side).

Conclusions:

- LC/CMS is a cost-effective IROA analysis approach for uniformly ^{13}C labeled amino acids.
- Tic Tac® example tablets can easily be distinguished using just two different enrichment levels of the same AA or using two different uniformly ^{13}C labeled AA, resulting in unique isotopic patterns and relative abundances.
- Initial studies of different enrichment levels suggest that LC/CMS can distinguish 5% different enrichment levels which would translate into millions of serialization steps based on 20 amino acids and 20 enrichment levels.

Literature and Acknowledgements:

[1] US Patent 8,969,251 Generation and use of isotopic patterns in mass spectral phenotypic comparison of organisms.
[2] US Patent 8,536,520 Method for generation and use of isotopic patterns in mass spectral data of simple organisms.
[3] Eikel D, Prosser SJ and Henion JD: Determination of amino acids using a novel liquid chromatography compact mass spectrometry system (LC/CMS) with mixed-mode separation media. 63rd Meeting of the American Society for Mass Spectrometry (ASMS) 2015, St. Louis, MO, USA
[4] Klecha L and Eikel D: Discovery Ion Current – A novel approach to plot total ion current real-time by enhancing signal from newly detected ions. 65th Meeting of the American Society for Mass Spectrometry (ASMS) 2017, Indianapolis, IN, USA

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