WEBINAR

Considerations for Chromatographic Method Development & Validations in Food, Beverages, Ingredients, Excipients, and Dietary Supplements

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AGENDA

- Introduction
- Review of Part 1 of our Webinar Series
- Method Considerations
- Instrumentation Platforms
- Case Studies
- Final Notes

Why do we validate analytical methods?

Method validation demonstrates that an analytical procedure is suitable for its intended use.

A validation creates documented proof that a lab(s) can analyze (quantitate, detect, etc.) an analyte(s) in the matrix(ces) of interest while meeting performance criteria (e.g. accuracy, precision, etc.) to the scope that has been deemed necessary.

Reliability or lack of reliable of data produced from an analytical method can have widespread impacts on health and safety!

Who relies on these methods?

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Part 1: Analytical Method Validation for Regulatory Compliant Testing

TERMINOLOGY & DEFINITIONS

VALIDATION

Establishes the performance parameters and suitability of a new or single-laboratory validated method.

MATRIX EXTENSION

Extensions of the scope of a validated method require a supplemental validation.

METHOD MODIFICATION

Changes to a method resulting from alternate equipment or procedures may require validation.

VERIFICAION

Ensures that a standard or "Official" method is implemented with equivalent performance as previously established in the validation.

METHOD FITNESS

Confirming that a method performs suitably for a specific matrix.

CHANGE CONTROL

Changes in laboratory personnel, suppliers and equipment require assessments to demonstrate equivalency.

When should methods be validated or verified?

Validation

- Development of a new method
- Extension to the scope of a previously validated method
- Modification of equipment, procedure or technique

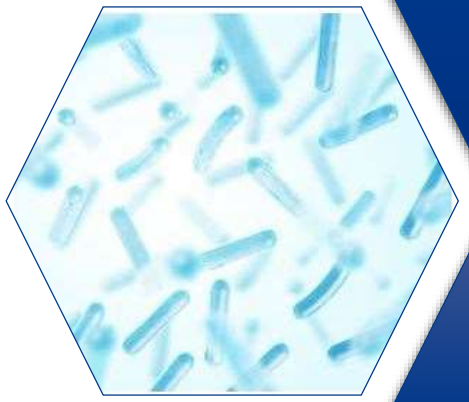
Verification

- Confirm method applicability to a specific matrix
- Demonstrate Proficiency with a newly implemented method
- Changes in equipment, components, personnel or suppliers

Who regulates method validation?

US FDA

ISO



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USP

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WHERE DO WE START?

Determine Method Requirements:

- Assay vs. Impurities?
- Qualitative vs. Quantitative?

What does this impact?

- **Detection limits**
- **Calibration**
  - **Single point**
  - **Calibration curve**
  - **Calibration Range**
- **Required suitability**

WHAT IS BEING TESTED?

Determine Properties of the Analyte

- Volatile or non-volatile?
- UV active?
- Fluorescent?
- Contains phosphorus/ halogen?

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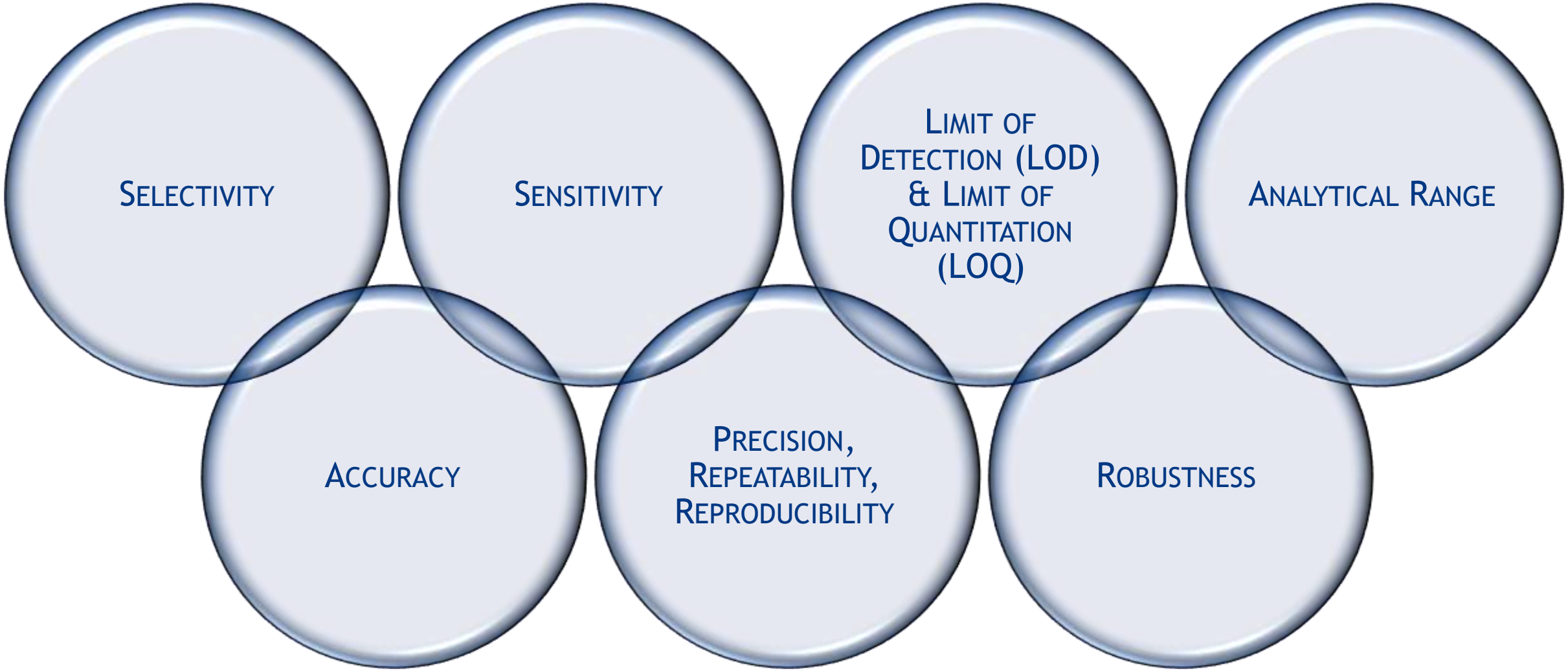
WHAT CRITERIA SHOULD YOU EVALUATE?



WHAT'S NEXT?

Develop the method:

STARTING POINT

-Similar method that is already validated.
-Literature sources
-Compendial method
-Institutional knowledge

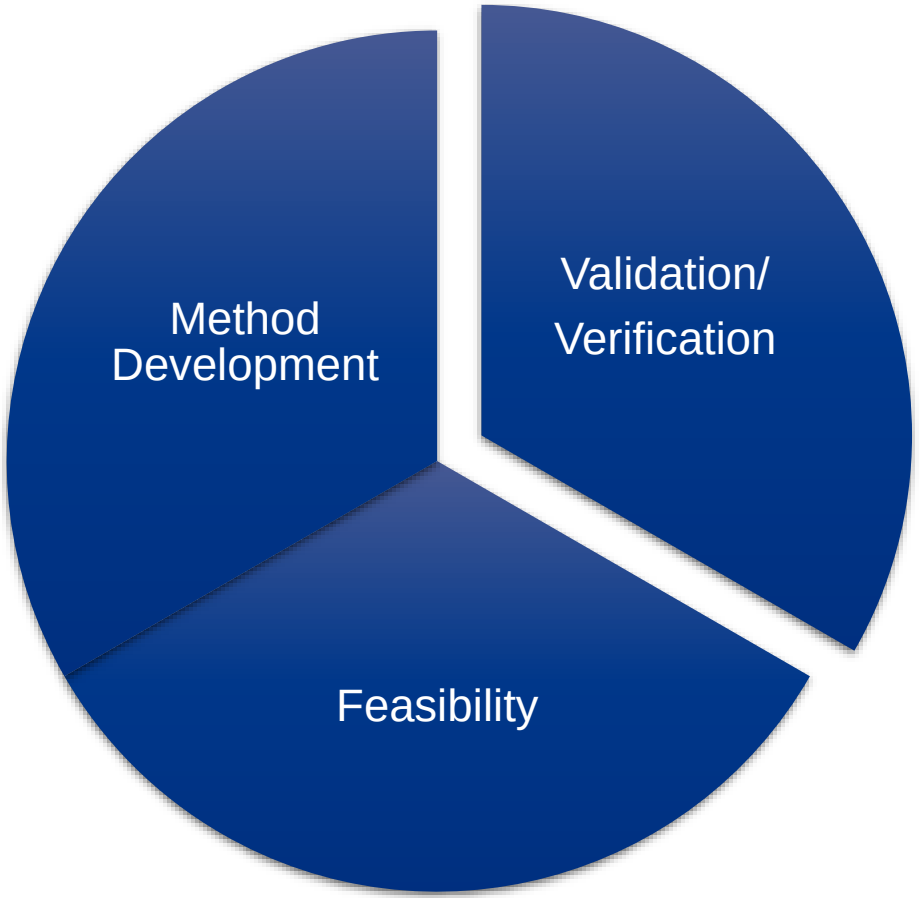
FEASIBILITY

Prior to any validation or verification, it is important to run feasibility to determine how the method performs and to identify potential issues. This often includes:
-detection limit testing
-spiking studies
-calibration range

WRITING YOUR PROTOCOL

Once you are confident that your method performs as you require, write your validation protocol. Rely on resources such as those provided by AOAC and the FDA to determine your passing criteria. Must define criteria *before* starting validation.

Now, Validate!



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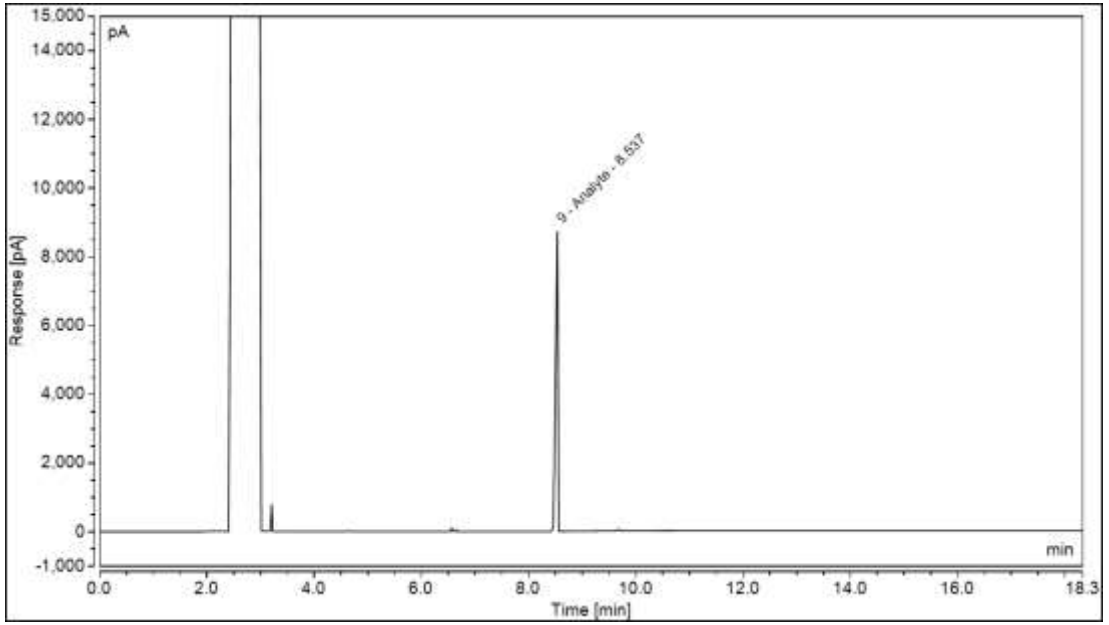
Gas Chromatography

Flame Ionization Detection (GC-FID):

Carbon containing analytes are combusted in a hydrogen flame and produce ions which are measured.

Pros	Cons
✓ Widely Available	✓ Not selective
✓ Universal Detector (for organic compounds)	✓ Not as sensitive as targeted detectors
✓ Reliable	
✓ Sensitive	
✓ Wide linear range	



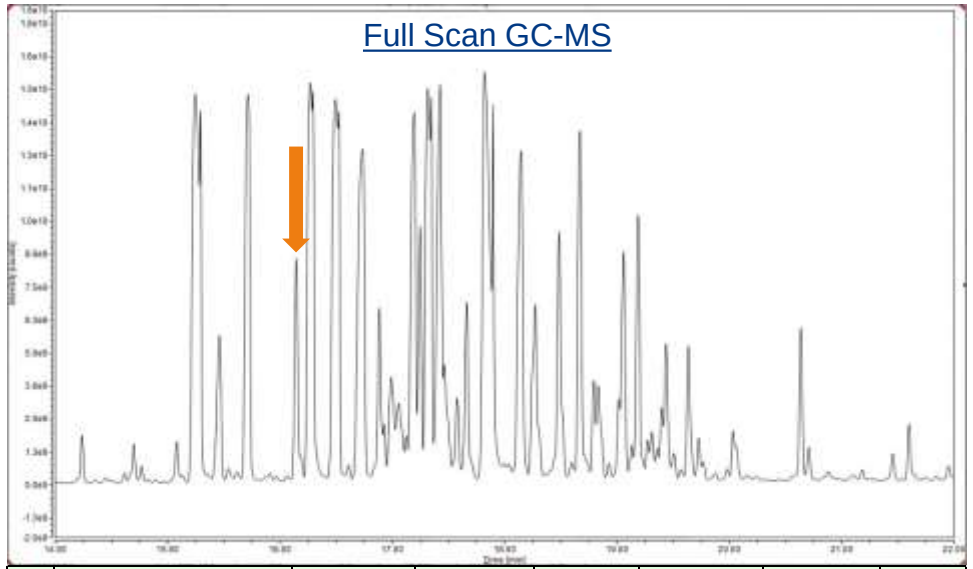
Gas Chromatography

Mass Spectrometry (MS or MS/MS):

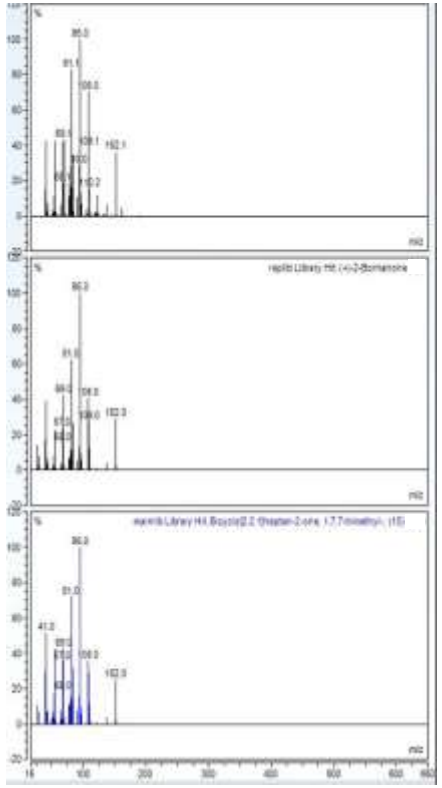
Compounds are ionized and separated and identified by mass.

Pros	Cons
✓ Sensitive	✓ Sensitive
✓ Universal Detector	✓ Universal Detector
✓ Selective	
✓ Can be used for identification	

Full Scan GC-MS



No.	Name	CAS Number	Mol. Weight	SI	Retention Time min	Area counts*min	Rel.Area %
43	Longifolene	475-20-7	204	854	16.034	47118297.5	0.08
44	1,2,4-Methenoazulene, decahydro-1	1137-12-8	204	841	16.071	57765842.6	0.10
45	(+)-2-Bornanone	464-49-3	152	900	16.175	775344200.3	1.29

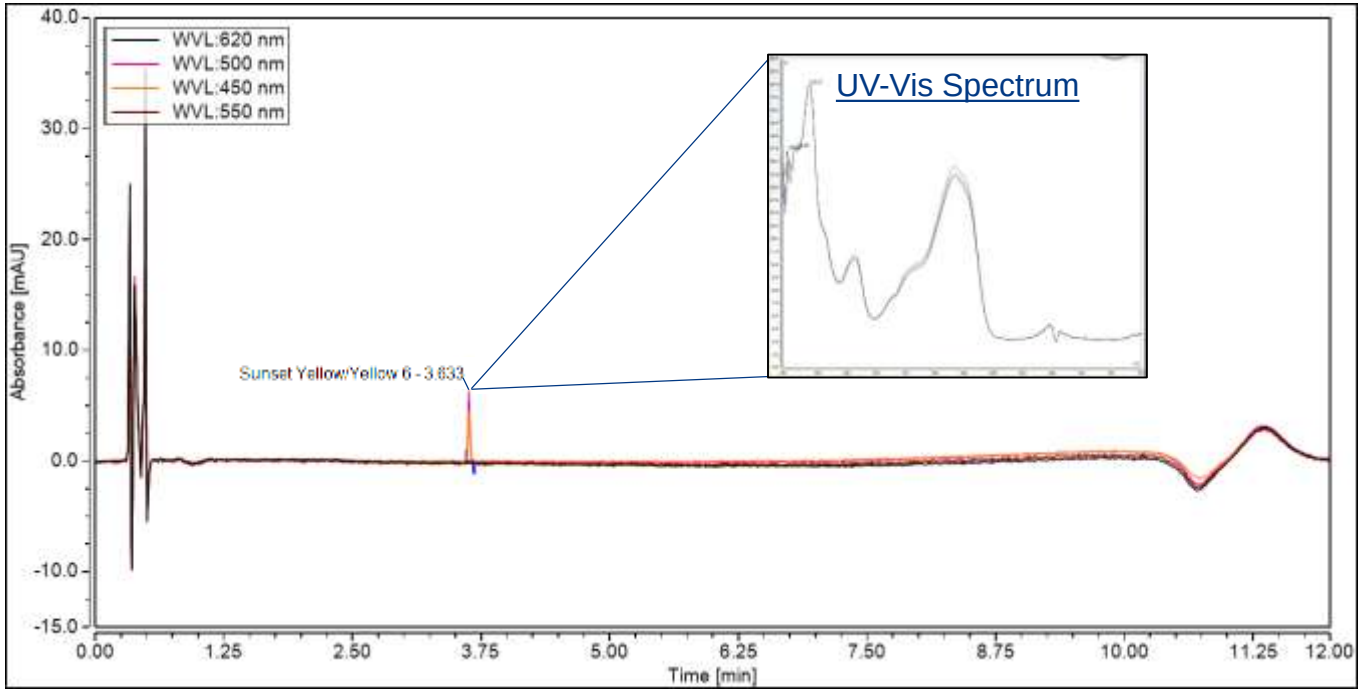


Liquid Chromatography

UV-vis/Photodiode Array (PDA):

Compounds containing chromophores absorb light at specific wavelengths.

Pros	Cons
✓ Sensitive	✓ Analyte must contain a chromophore
✓ Targeted detector	
✓ Selective	
✓ Can be used to aid in identification (PDA)	
✓ Wide Linear range	
✓ Very Common/ Inexpensive	

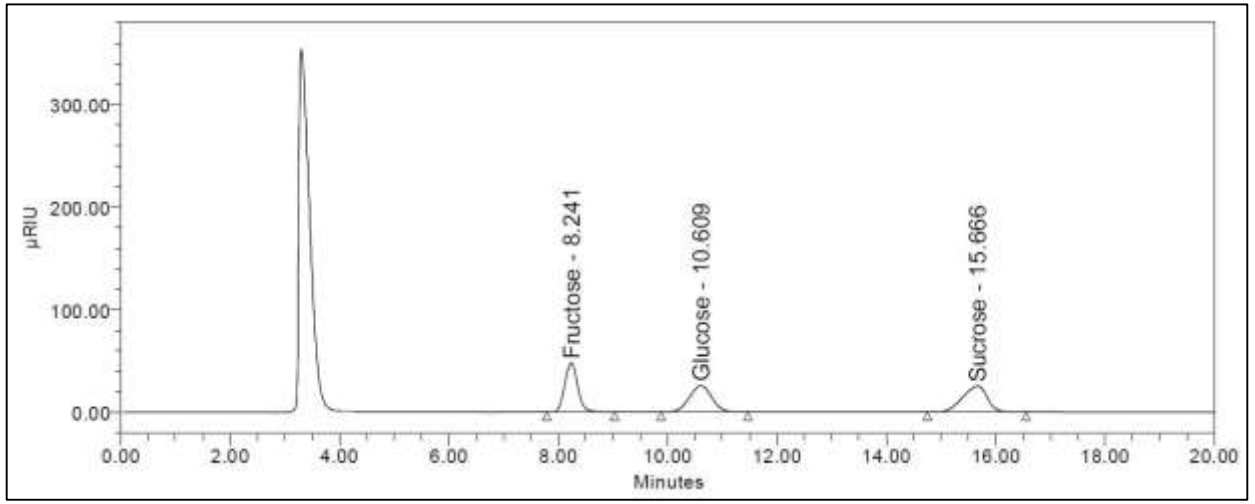


Liquid Chromatography

Refractive Index (RI):

The refractive index of a reference cell containing the mobile phase is compared to the refractive index of the mobile phase passing through the column. The presence of analytes results in a change in refractive index.

Pros	Cons
✓ Universal detector	✓ Low sensitivity
✓ Inexpensive/common	✓ No selectivity
	✓ Cannot use gradients
	✓ Sensitive to environment
	✓ Matrix interferences

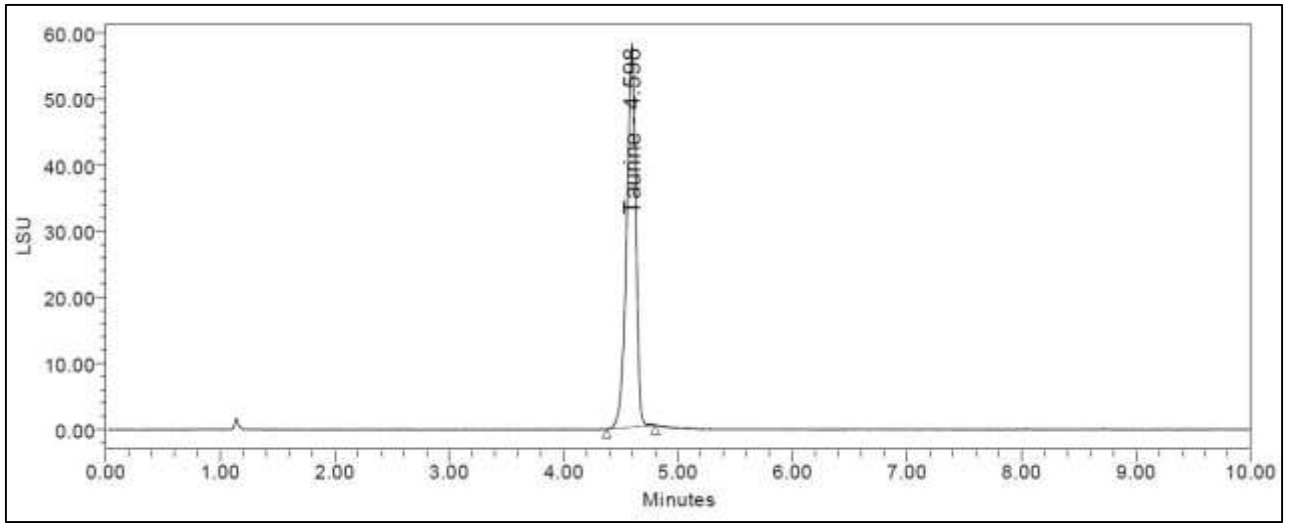


Liquid Chromatography

Evaporative Light Scattering Detector (ELSD):

The eluent is evaporated, and the resulting analyte particles are passed through a beam of light, and the amount of scattered light is measured.

Pros	Cons
✓ Sensitive	✓ Non-volatile buffers can't be used
✓ Universal Detector	✓ Narrow linear range
	✓ Sensitive to matrix components

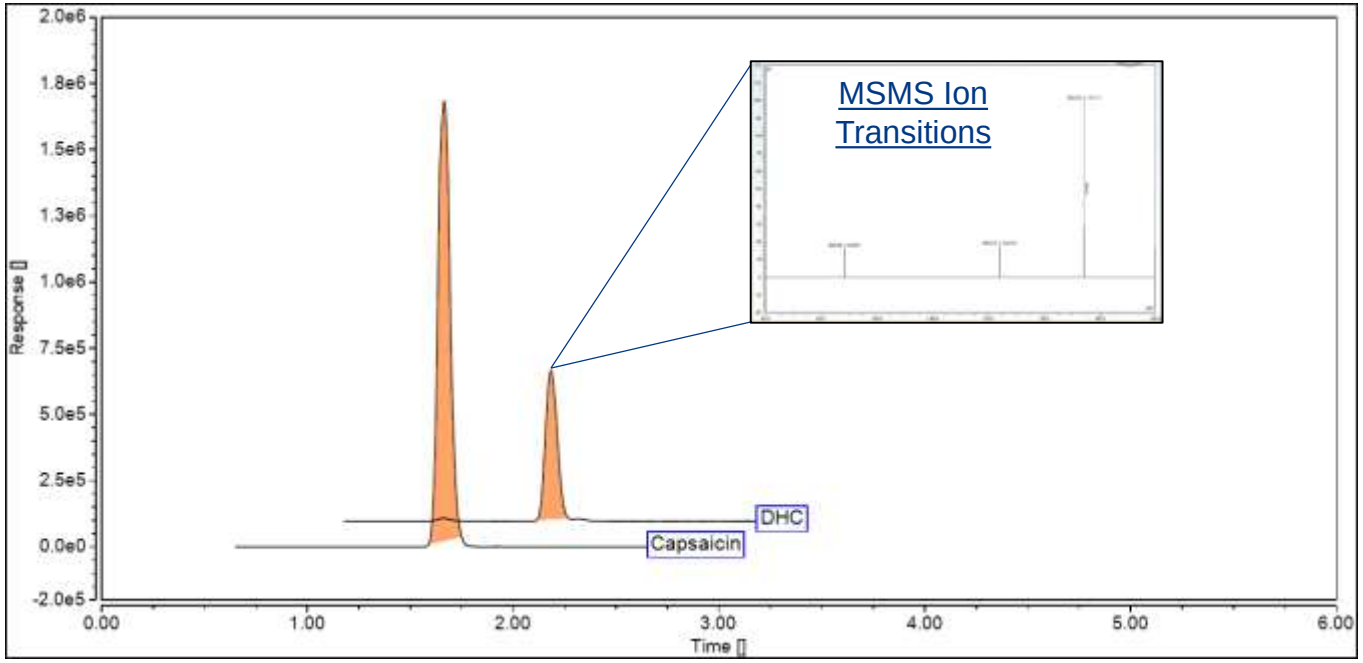


Liquid Chromatography

Mass Spectrometry (MS or MS/MS):

Compounds are ionized and separated by mass. Many different ionization and mass separation techniques are available.

✓ Pros	✓ Cons
✓ Sensitive	✓ Non-volatile buffers can't be used
✓ Universal Detector	✓ Sensitive to matrix components
✓ Selective	✓ Expensive
✓ Can be used for identification	✓ Complex setup and analysis
	✓ Complex maintenance



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CASE STUDY

Analysis of a Novel Health Molecule in a Smoothie Beverage Formulation

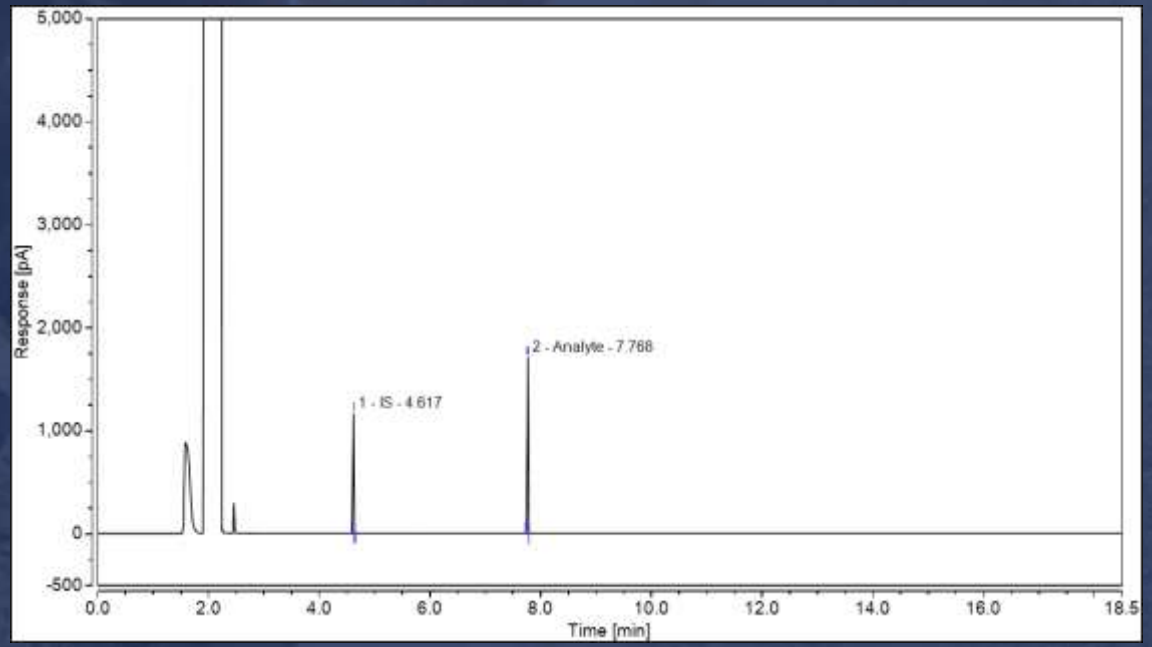
Considerations:

- *Quantitation at weight% level in product (assay)*
- *Complex smoothie matrix*
- *Novelty of molecule*

APPROACH

- Based on assessment of the molecule's structure (e.g. not UV active, good volatility) and with the goal of keeping analysis cost effective, GC-FID was selected as a platform for method development.

- Feasibility studies included:
  - Optimization of instrument conditions to achieve separation (from IS), good peak shape, and the desired detection level.
  - Testing of different solvents for standard preparation and sample extraction.
  - Testing of multiple internal standards (IS).
  - Sample spiking studies to assess recovery throughout the procedure.



CHALLENGE

- Matrix complexity
  - Obtain blank smoothie matrix with no active ingredient for use during validation spiking studies.
  - Optimize extraction using organic solvents to reduce extraction of interfering substances.

- Standard sourcing challenges due to compound novelty
  - GC-MS analysis for confirmation of structure of client provided standard used for specificity during validation.

CASE STUDY

Analysis of a Trace Impurity in an Artificial Sweetener

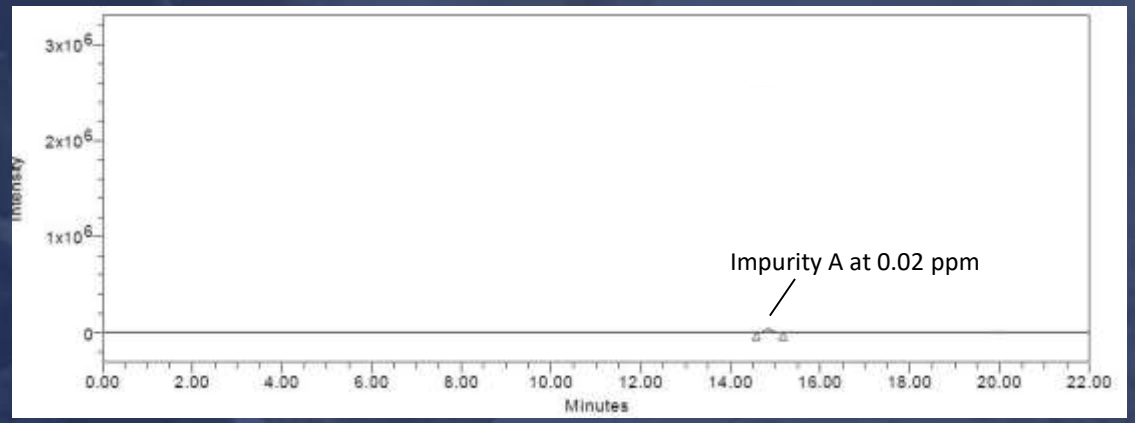
Considerations:

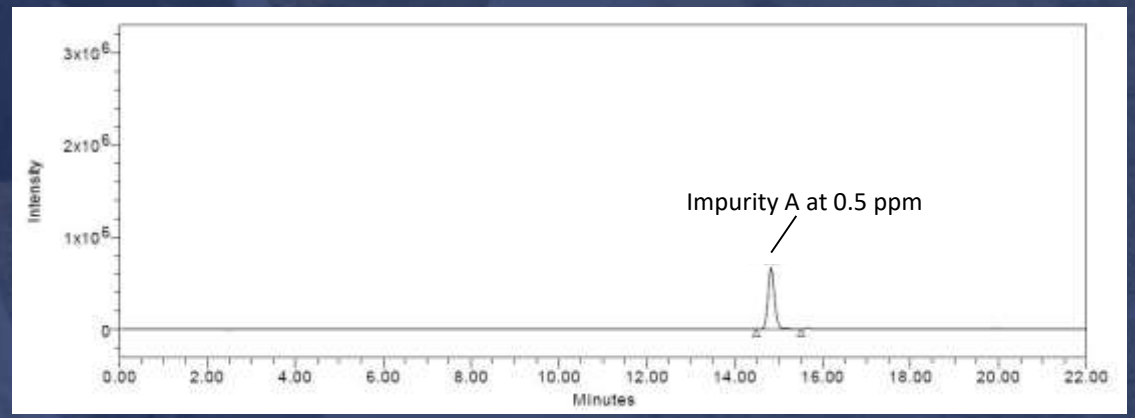
- *Quantitation at low level (<0.1%) in product (impurities)*

APPROACH

- Based on the molecule's structure and expected concentration, LC-MS was selected as a platform for method development.

- Feasibility studies included:
  - Optimization of instrument conditions to achieve retention and good peak shape.
  - Optimization of mass spectrometry parameters to achieve the desired detection level for the analyte.
  - Sample extraction testing to determine whether the sample material could interfere with trace analyte detection.





CHALLENGE

- Initial sensitivity challenges due to ionization mode for analyte (negative)
  - Detailed optimization of single ion monitoring (SIM) conditions (e.g. cone voltage) allowed for improved detection.

- Due to the nature of SIM LC-MS analysis, the analyte was found to fluctuate leading to differences in linear range and signal intensities between analytical runs.
  - Validation criteria and the routine SOP were written to fit the purpose of the analysis while allowing for day-to-day flexibility in instrument intensity.

CASE STUDY

Analysis of 34 Trace Drug Residues in Wastewater

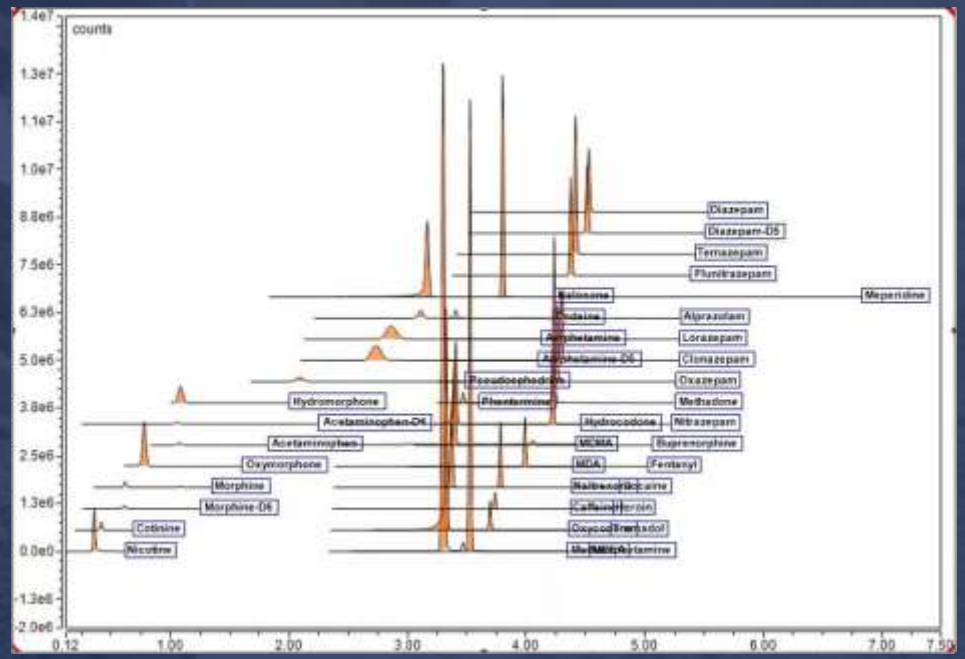
Considerations:

- *Trace quantitation and detection (ppb levels)*
- *Large number of analytes of interest from a variety of structural drug classes*

APPROACH

- Based on the large number of analytes, their variation in structures, and the desired level of detection, the most promising platform was LC-MSMS.

- Feasibility studies included:
  - Identifying and optimizing unique MSMS transitions for each analyte with clean baselines to ensure low detection and high specificity.
  - Testing of a variety of isotopically labeled internal standards for each drug class.
  - Chromatographic method adjustment to obtain detectable peaks with shape and separation optimized for as many analytes as possible.



CASE STUDY: Analysis of 34 Trace Drug Residues in Wastewater

CHALLENGE

- Method development for a large number of analytes.
  - MSMS transitions, internal standard, etc. need to be adjusted individually.
- Determining validation guidelines suitable for a large number of analytes.
  - EPA and FDA recommendations were carefully considered to ensure the method purpose was being met with concessions for the low level quantitation.
    E.g. Replicate spikes were performed at 10 ppb.

Opiates
Methadone
Morphine
Oxycodone
Oxymorphone
Tramadol
Naloxone
Naltrexone
Meperidine
Heroin
Buprenorphine
Codeine
Fentanyl
Hydrocodone
Hydromorphone
Meperidine

Benzodiazepines
Alprazolam
Clonazepam
Diazepam
Flunitrazepam
Lorazepam
Nitrazepam
Oxazepam
Temazepam

Amines
Amphetamine
Methamphetamine
Phentermine
MDA
MDEA
MDMA
Cocaine

Other/OTC
Pseudoephedrine
Cotinine
Acetaminophen
Caffeine
Nicotine

CASE STUDY

Identification of Nine FD&C Approved Dyes

Considerations:

- *Qualitative identification i.e. presence or absence*
- *Many possible applications*
  - *Matrix extension(s) likely necessary*

APPROACH

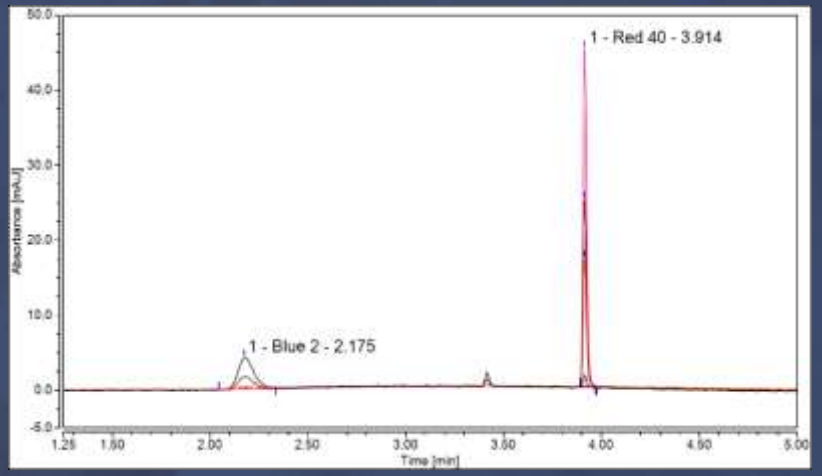
- Based on the analyte properties (i.e. low volatility dyes), and the desire to apply the method to a variety of extracts and samples, LC-UV was selected as the platform.
- Feasibility studies included:
  - Chromatographic method optimization to ensure clear separation and identifiable UV-VIS spectra for each analyte.
  - Test extractions and purifications of a variety of food substances with known ingredients.

CASE STUDY: Identification of Nine FD&C Approved Dyes

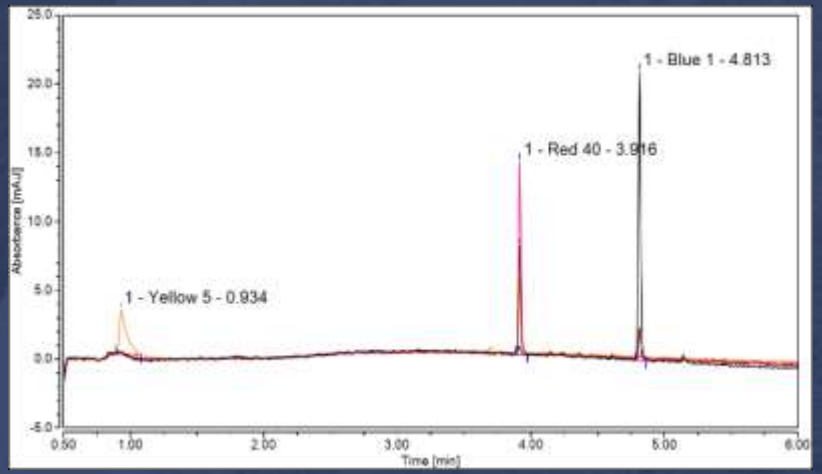
CHALLENGE

- Method development with possibility of many future applications in a variety of matrices.
- Validation guideline determine for a single lab qualitative method:
  - EPA and FDA recommendations were carefully considered to ensure the method purpose was being met with flexibility for future extensions.
    - Blind spikes were used to demonstrate specificity and ability to identification each dye.

Grape Candy



Ranch Flavored Corn Chips



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GUIDANCE AND REFERENCES

- ✓ Analytical Procedures and Methods Validation for Drugs and Biologics, CDER, CBER, 2015, www.fda.gov.
- ✓ AOAC Guidelines for collaborative study procedures to validate characteristics of a method of analysis, 2002, www.aoac.org.
- ✓ ISO Guide 33:2015 Reference materials — Good practice in using reference materials, www.iso.org.
- ✓ ISO/IEC 17025:2015 General requirements for the competence of testing and calibration laboratories, www.iso.org.
- ✓ ISO 21748:2010 Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation, www.iso.org.
- ✓ Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Edition, www.fda.gov.
- ✓ Guidance for Industry #118, Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues, US FDA CVM, 2003, www.fda.gov.
- ✓ Validation of analytical procedures: Text and methodology Q2(R1), 2005, www.ich.org.
- ✓ USP <1224> Transfer of Analytical Procedures, https://www.usp.org
- ✓ USP <1225> Validation of Compendial Procedures, https://www.usp.org
- ✓ USP <621> Chromatography, https://www.usp.org

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