The Development of a Novel LC-MS/MS Method for the Detection of Four Laxatives in Urine

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Aims:

- To **develop and validate** a new method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect four laxatives in urine

- To **compare** the new method to the current high performance thin-layer chromatography (HPTLC) method in routine use at Salford Royal Hospital
Laxatives

- Natural or synthetic compounds that promote bowel evacuation
- Used to clear the bowels prior to a medical/surgical procedure and in the treatment of constipation
- Four groups:
  1. Bulk-forming
  2. Osmotic
  3. Faecal softeners/lubricants
  4. Stimulants

- Desacetyl bisacodyl
- Phenolphthalein
- Rhein
- Dantron
Why Measure Laxatives?

- Laxatives may be misused or abused:
  - Eating disorders
  - Habitual
  - Factitious disorders

- Guidelines (Gut, 2003) recommend that laxatives should be measured in urine of individuals with unexplained chronic diarrhoea
Current Method - HPTLC

- Current method uses high performance thin-layer chromatography (HPTLC)

1. Samples applied to gel
2. Gel placed in liquid
3. Liquid moves up the gel
4. Laxatives move up gel depending on affinity to gel and liquid
Why Do We Need A New Method?

- Problems with current method:
  - Difficulties with interpretation
  - Long analysis time with lots of steps
  - Use of very old equipment that cannot be repaired
  - False positives and false negatives!!

Positive or negative??

Decreasing concentration
Extraction of laxatives from urine into a solvent

Separation using liquid chromatography (LC):

Detection using mass spectrometry (MS/MS):

- Uses mass and electrical charge to identify and quantify the laxatives
Validation

- Does the method work correctly every time?

- Includes:
  - **Accuracy** – how close the measured concentration is to the true concentration
  - **Precision** – how close results are to each other
Method Comparison

- Compared analytical **sensitivity** of methods:
  - Lowest concentration the method can measure
  - New method can measure lower concentrations than the current method

- Analysed 47 patient samples:
  - 37 Not detected using current and new method
  - 7 Detected using current and new method
  - 3 Detected using new method
  - Not detected using current method
Why Is This Method Novel?

- Most published methods are very old HPTLC methods

- Very few published LC-MS/MS methods and none that measure all four laxatives within the same method!
Conclusion

- A LC-MS/MS method that detects four laxatives in urine has been developed and validated.
- The new method has several advantages over the current method:
  - Greater sensitivity
  - Shorter analysis times
  - More easily interpretable results
- The method is novel as it:
  - Measures all four urine laxatives within the same method.
Thank you for listening

Any questions?