

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



Heather Wilshaw


Trainee Healthcare Scientist

Salford Royal Foundation Trust



MSc Project

- 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
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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**

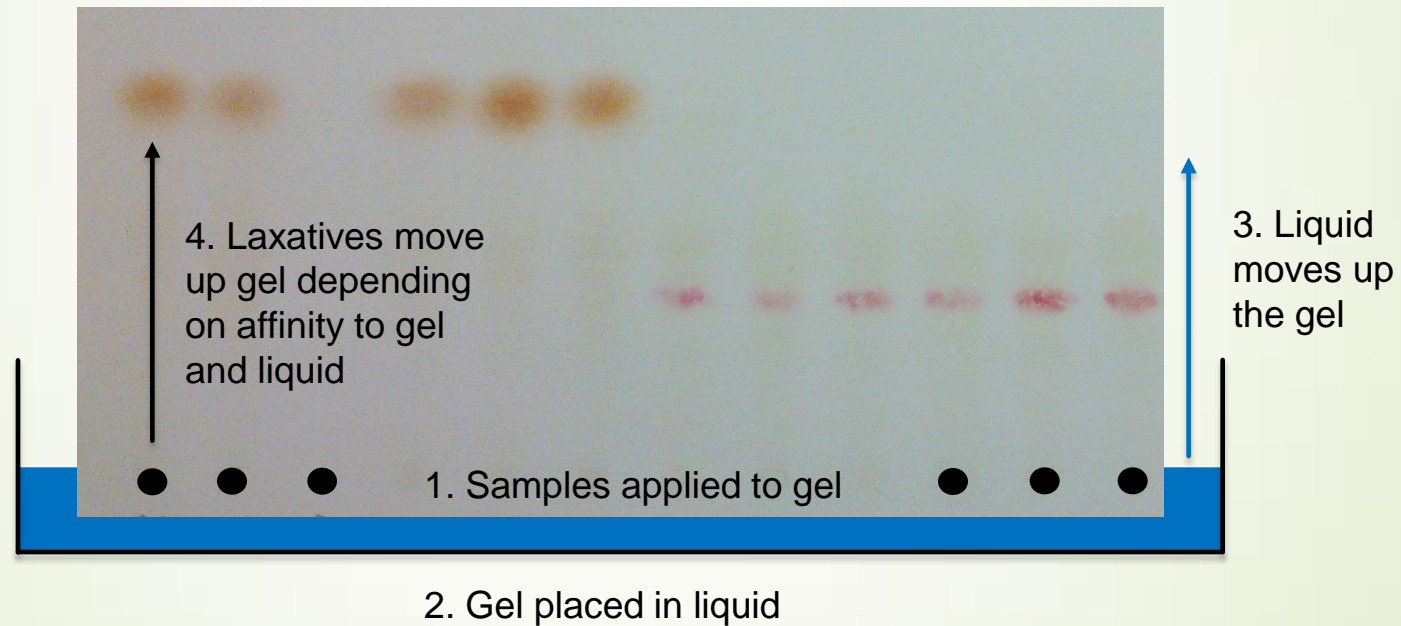


Why Measure Laxatives?

- ▶ 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)



Why Do We Need A New Method?

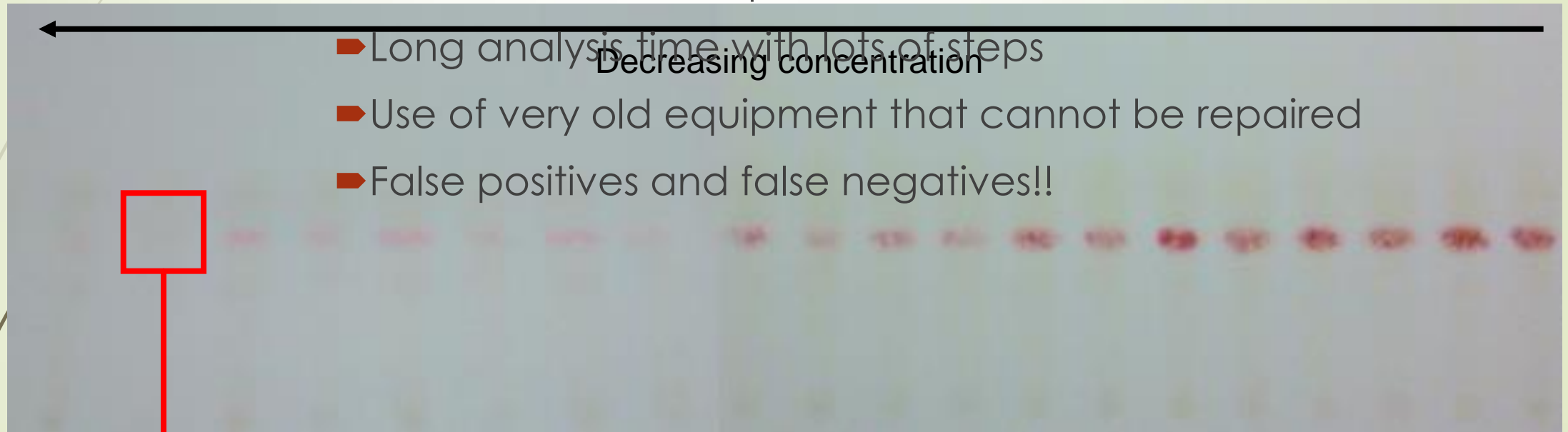
- Problems with current method :

- Difficulties with interpretation

- Long analysis time with lots of steps

- Use of very old equipment that cannot be repaired

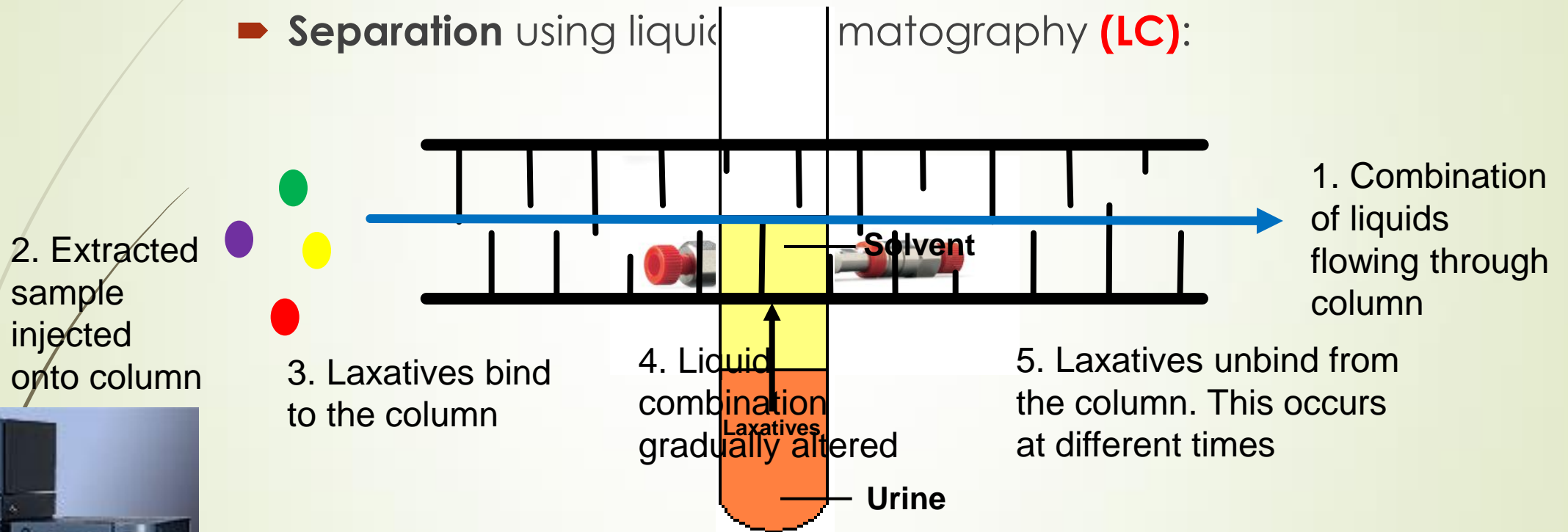
- False positives and false negatives!!



Positive or negative??

Method Development – LC-MS/MS

- **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



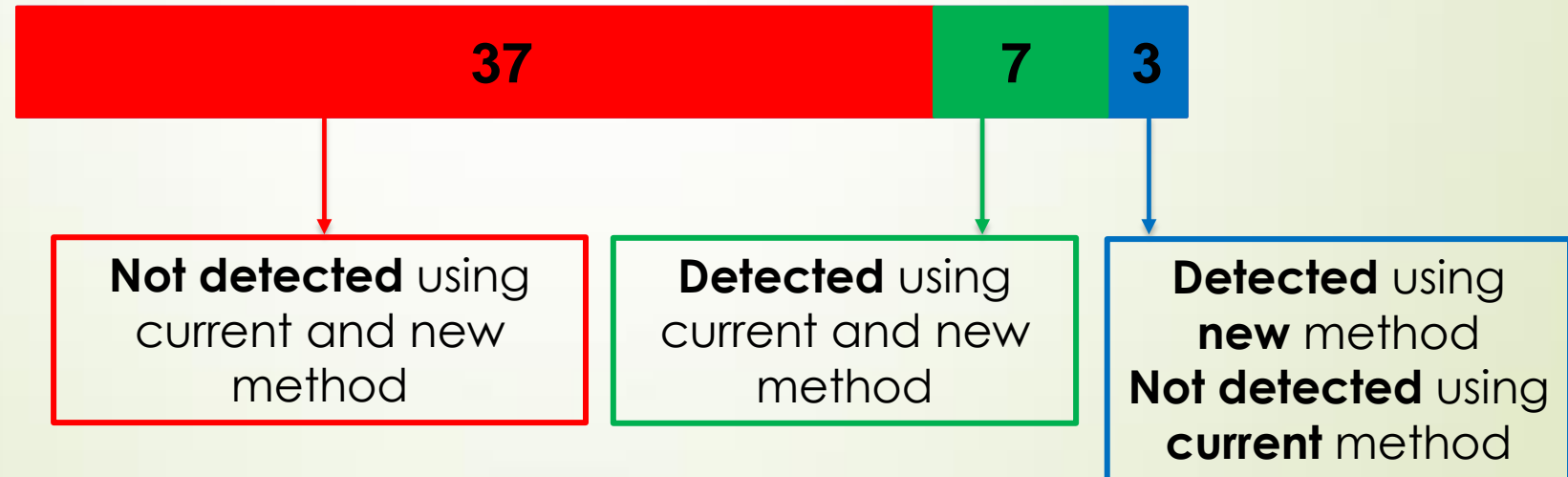


Validation

- **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
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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:





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?

