Tales of the Unexpected.

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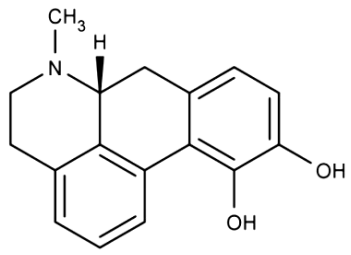
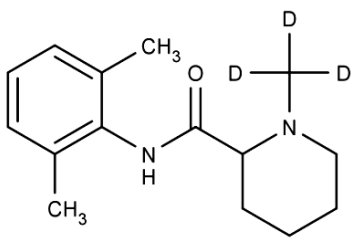
First tale

We developed and validated a method for the quantification of apomorphine in human plasma. The method used deuterated analogue mepivacaine as the internal standard.



Apomorphine



Mepivacaine-d_3

Method

The method used a protein precipitation procedure with acetonitrile. The chromatography was an Ace Ultracore SuperPhenylHexyl column. The calibration range was 0.02 to 10.0 ng/mL. When we received real samples, this is what we saw in the apomorphine MRM.

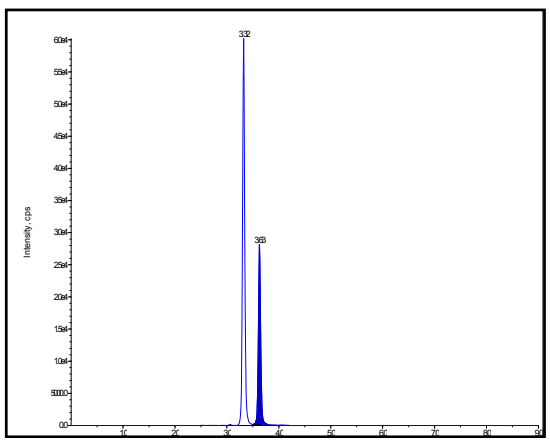
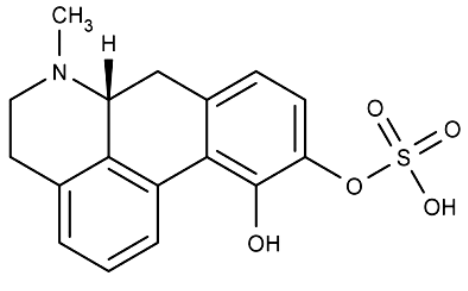


Figure 1: Apomorphine MRM chromatogram of an extracted clinical trial sample showing extra peak.

The peak eluting before the apomorphine was identified as the mono sulphate metabolite of apomorphine. This was not observed in the preclinical studies. The sponsor was then requested by the regulatory authorities to synthesise reference material for this metabolite so that the method could be revalidated to quantify the metabolite and parent.



Apomorphine mono sulphate

Luckily we still had some clinical samples in the freezer so that we could work out required calibration range for metabolite. We knew from the ISR results for apomorphine in the previous study that the mono sulphate appeared stable in plasma.

New method to be validated was apomorphine over a concentration range of 0.04 to 20 ng/mL and the mono sulphate with a range of 1 to 500 ng/mL.

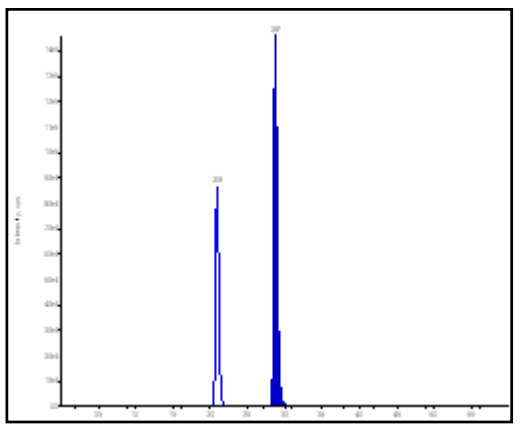
Issues during revalidation

- **The apomorphine sulphate contained apomorphine so the spiking solutions had to be corrected for this.**
- **The mixed spiking solutions were prepared in 0.1/99.9 w/v ascorbic acid/0.1M hydrochloric acid. These agents were required to stabilize the apomorphine in solution but the mono sulphate was unstable under these conditions.**
- **Separate spiking solutions had to be prepared**

Second tale

Method for compound-X and 3 metabolites was set up and validated in human plasma after supporting toxicity studies in rat and monkey.

Figure 2: MRM Chromatogram of the parent from a clinical sample showing extra peak.



This peak was identified as the N-glucuronide metabolite. Eventually discovered that not all genotypes produce this metabolite hence why it was not seen in the *in vitro* human metabolism studies.

The reference material of the metabolite was synthesised and it was discovered that regardless of its intensity to the parent in the parent MRM above it was actually present at levels ten times greater than the parent!

The reference material of the glucuronide metabolite was supplied as a solution. Due to its concentration in the solution and the concentration required in plasma the solution had to be evaporated under nitrogen prior to mixing with the parent and other metabolites so that the concentration of solvent when spiking plasma was less then 2%. Unlike the apomorphine and mono sulphate these mixed solutions were stable.

The new method was validated over a concentration range of 5 to 1000 ng/mL for the parent drug and 3 metabolites. Whilst the N-glucuronide concentration range was 50 to 50,000 ng/mL.

Extra comments

- **So what would the regulatory authorities have done if these metabolites had not been fragile in an API source?**