B46924 pharm-analyt Nitrite Determination

In a discussion with a costumer about Nitrosamines the determination of nitrite came up. Nitrite is the direct precursor in the formation of Nitrosamines and its presence in raw material or water in the manufacturing process of pharmaceutical drugs and solutions should be avoided by all means.

Nitrite determination with LC-MS/MS is however difficult. Its mass is 46 amu and it is therefore **not** in the accurate mass determination window of the Orbitrap XL we have in house but the ion trap only which is definitely a drawback in regards to loss of selectivity (mass window of ± 0.5 usable only, not 0.005 or less). And of course, the transmission for such a low mass is not good either, it was clearly seen that for potassium for example (39 amu) the transmission is still halfway ok but definitely not good, but for sodium (23 amu) it is very poor. When using a Triple-Quad System (Xevo TQ-XS) there is additionally the drawback that typically fragmentation should be used in order to reduce chemical background and hence improve sensitivity. Well, for nitrite extremely small fragment ions at 30/31/32 amu could be found, however fragmentation yield or respectively fragmentation ion transmission yield at mass 30/31/32 amu is very poor. So, a "Pseudo"-MRM transition 46-46 m/z had to be used. Since however the NO_2 is an actual molecule and not an atom the collision energy used had to be small; this is another drawback, for a Cl^- atom-ion for example the collision energy used could be set at a much higher voltage which then also could reduce chemical background in the chromatogram and hence enhance sensitivity (since Cl^- is an "undestroyable" atom-ion and not a molecule-ion).

The attempt was done to look for or generate nitrite-adduct ions which would then firstly show greater mass (resulting in higher ion transmission yield and sensitivity) and secondly provide better conditions for fragment ion formation and fragmentation yield. The mobile phase used contained a lot of ammonium acetate, however only on the Orbitrap a halfway intense nitrite-acetic-acid-adduct ion could be found, not on the XEVO TQ-XS. Another approach was to use low mM concentration of Cs^+ ions which is sometimes used in e.g. sugar determination with LC-MS/MS. Cs ions would work for e.g. Cl^- determination $[Cl^-Cs^+_2]^+$ very nicely and strongly improving sensitivity for it, even for some other inorganic acids. However for nitrous acid only very poor ion yield of $[NO_2^-Cs^+_2]^+$ could be found. So direct determination appeared to be the only choice.

Another difficulty when determining nitrite with LC-MS/MS is that nitrate interferes. Nitrate ions are basically ubiquitously present, in pipette tips, autosampler vials, mobile phases etc. And nitrate – even when using ESI as a very soft ionisation technique – degrades in the ion source to nitrite. Although both substances are chromatographically separated it still interferes and elevates the baseline for nitrite strongly. When leaving acetonitrile in the mobile phase out, the baseline could be lowered by about 5 times using aqueous conditions only. ASTM-I water (from inhouse system) appeared to be less contaminated then regular HPLC water. When pipette tips, autosampler vials etc. were washed out directly with ASTM-I water sensitivity was best respectively blank values were lower than without pre-washing. The use of a special nitrate-free water would most likely result in even better sensitivity.

Direct nitrite determination with ESI in negative ion mode on the Trinity P1 column was most sensitive when a higher pH value around 6-7 was used compared to pH 3-4 on the LC-MS/MS system.

At last UNISpray was used showing about 5 times better sensitivity than regular ESI, it could well be that part of the improved sensitivity result from less degradation of nitrate to nitrite during this ionisation process. With this system an LLOQ of about 200 ng/L could be reached.

Then the idea of a different detector came up, one, that may not be prone to interference from nitrate at all. **Electrochemical Detection!** With HPLC-Electrochemical Detection it became very quickly clear that nitrate is not or practically not oxidizable, meaning, the signal response of nitrate is only about 0.05 % compared to nitrite; it is practically not visible with this detector. Lower pH is much better for sensitivity for EC detection, a pH of 4 showed a huge improvement compared to pH 6-7. Selectivity was tested a little, alkaline ions show no response at all at the voltage settings used for nitrite determination. Chloride ion show a small peak, bromide however looks like being more readily oxidizable.

Currently about 50 ng/L could be reached with this detection system, however there are still improvements possible that would very likely lead to an even better sensitivity yet. These improvements would be changing to a **strong** anion exchange column instead of the **weak** anion exchange column. Then a lower pH mobile phase could be used for elution and hence quite likely a better sensitivity, see above. And assembling the HPLC system with a pre-oxidation chamber between HPLC-pump system and the auto-sampler. This pre-oxidation chamber would then be used to remove all oxidizable compound coming from the mobile phase (even nitrite) **before** entering into the auto-sampler. This then would lead to a lower baseline yet, hence, improved sensitivity.