Ultra-Trace Detection of NMBA in Liquid, Gel and Cream Formulations

RESOLIAN ANALYTICAL SCIENCES

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Background

N-nitroso compounds are considered to have extremely high carcinogenic potency, and several medications have been subject to recalls due to the presence of these impurities. Nitrosamines in pharmaceutical products can arise from Active Pharmaceutical ingredients (API), excipients, manufacturing processes, direct/indirect cross-contamination from solvents, and degradation during storage. To ensure the safety of these products, strict guidelines have been established by drug regulatory authorities for all drug products in the market.

N-nitroso-*N*-methyl-4-aminobutyric acid (NMBA, Figure 1) is a N-nitroso compound that has been known to be carcinogenic in animals and potentially a human carcinogen also. The EMA recommends an Acceptable Intake (AI) limit of 96.0 ng/day for NMBA.

Here, a method has been developed for the detection of NMBA in pharmaceutical liquids, creams and gels with the use of Hydrophilic interaction liquid chromatography- electrospray ionisation mass spectrometry (HILIC-ESI-MS/MS).

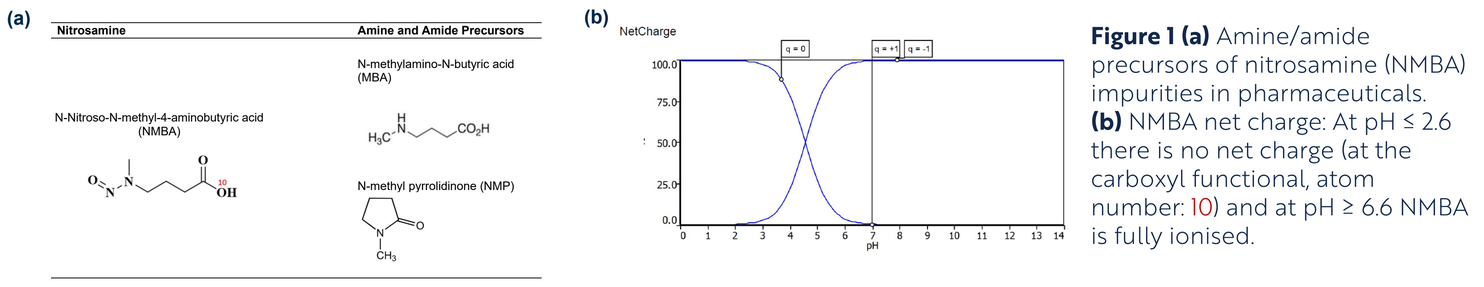
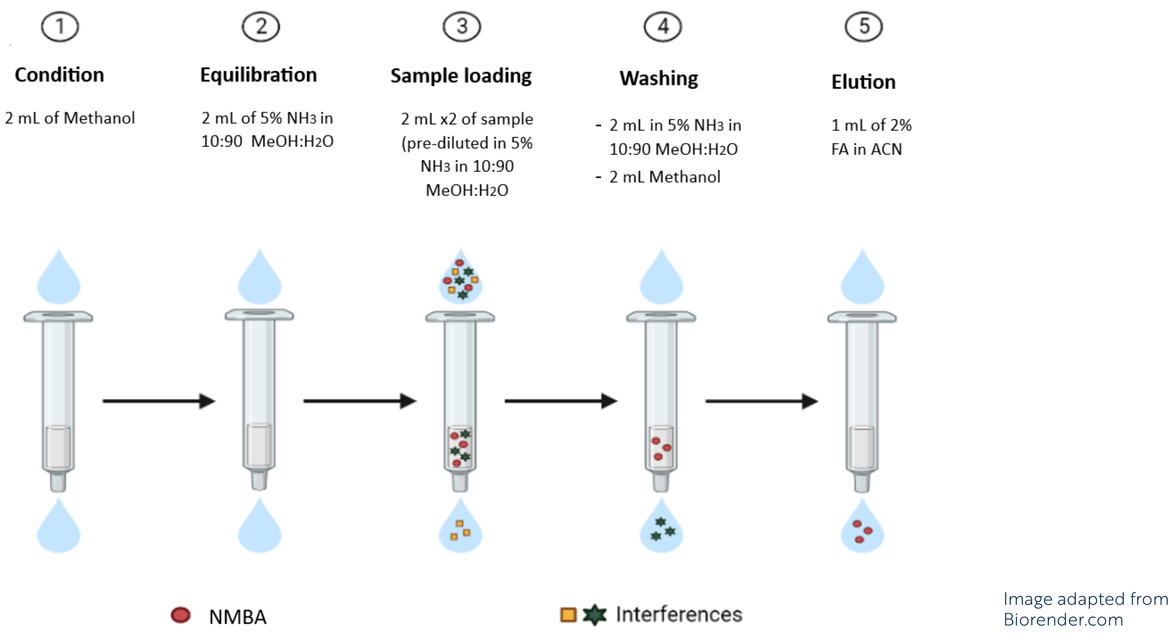


Figure 1 (a) Amine/amide precursors of nitrosamine (NMBA) impurities in pharmaceuticals. **(b)** NMBA net charge: At pH ≤ 2.6 there is no net charge (at the carboxyl functional, atom number: 10) and at pH ≥ 6.6 NMBA is fully ionised.

Extraction Procedure



Cartridge used:
Waters Oasis
3cc MAX

Image adapted from Biorender.com

LC Parameters

Instrument: Acquity UPLC
Column: BEH Z-HILIC
Column Temp: 40°C
Mobile phase A: 0.1% NH_3 in water
Mobile phase B: 0.1% NH_3 in ACN
Injection mode: Partial loop

MS Parameters

Instrument: API 4000
Ionisation: ESI
Scan type: MRM
Polarity: Negative
TEM: 300°C
EP: -10.0
GS1: 60.0
GS2: 30.0
IS voltage: -4000
CUR: 30.0
CAD:10.0
MS acquisition time: 5 mins

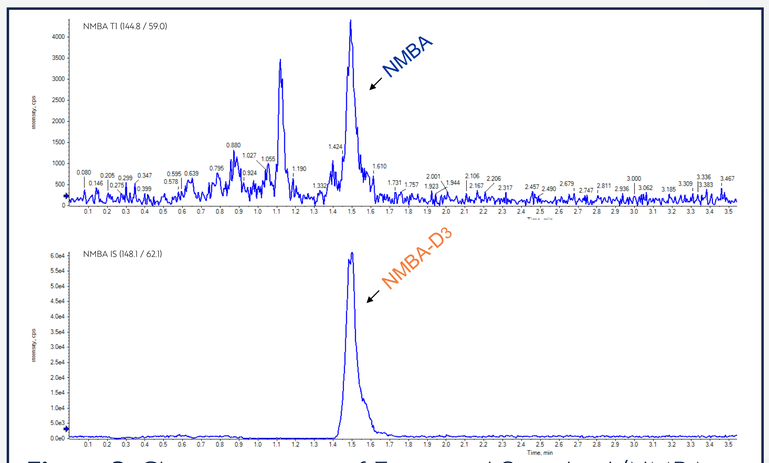


Figure 3. Chromatogram of Extracted Standard (NMBA at 0.8 ng/mL and NMBA - D_3 at 10 ng/mL)

Summary

- NMBA was successfully extracted from a range of pharmaceutical products using ion-exchange cartridges.
- The developed high-pH HILIC methodology provided improved sensitivity for the detection of NMBA when compared to existing reversed phase methodologies.

Experimental Results and Discussion

Initially, reversed phase LC conditions were trialled but signal to noise (S/N) and % recoveries of spiked samples fell below criteria, therefore Hydrophilic interaction liquid chromatography (HILIC) was investigated under basic/alkaline conditions. Such conditions are not commonly used due to the concern of column instability but with the advancements of LC column technology, high-pH mobile phases are being used more frequently in LC/MS systems. HILIC provides various advantages, such as higher sensitivity when used with electrospray ionisation (ESI) and a lower back pressure compared to reversed-phase LC.

Two types of HILIC columns were investigated in method development; BEH Amide and BEH Z-HILIC. Good sensitivity was achieved with both HILIC columns, obtaining an LOQ of 0.8 ng/mL.

Challenges overcome: The BEH Amide column failed to retain a suitable retention time (RT) for NMBA under experimental conditions, therefore a Z-HILIC column was tested and proved successful at maintaining a robust RT of ~1.5 minutes, at a flow rate of 0.8 mL/min (Retention factor = 2.5).

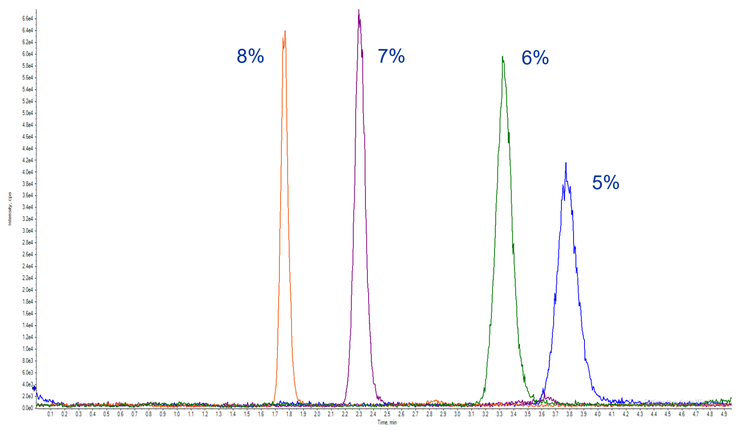


Figure 2. Chromatogram of scouting gradient of Non-Extracted Standard.

For HILIC, water is the strongest solvent and thus the aqueous elution strength should not be lower than 3%. At this percentage, an immobilised water layer can be formed on the stationary phase to create the hydrophilic partitioning mechanism. With this in mind, a starting aqueous elution strength of 3% was tested and incrementally increased to 8% (see Figure. 2). A dramatic reduction in RT was observed for the analyte for each 1% incremental increase in aqueous isocratic elution strength. The percentage that gave a good peak shape and ideal RT for NMBA was identified as 8%.

Both mobile phases had the addition of ammonia to give a pH ~10. The composition of ammonia in the mobile phases was initially tested at 1% but the final composition chosen was 0.1% ammonia to preserve the life of the column. Due to ammonia's volatile nature, the mobile phases were prepared fresh on the day of analysis.