Analysis of Nitrosamines Using Unique Stationary Phase Technology

Joseph J. Pesek, Maria T. Matyska, Tanya Hiltz, MicroSolv Technology Corporation

Nitrosamines are highly toxic and are suspected to be a human carcinogen. At high doses, it has been shown to be a hepatotoxin that causes liver fibrosis and cancer in several animal species. Due to the toxic nature of nitrosamines, these compounds must be monitored using reliable methods. Analysis of nitrosamines is particularly important in the pharmaceutical industry. An overview of methods developed for nitrosamine analysis has been published [1] as well as a more specific report on the use of supercritical fluid chromatography [2]. Nitrosamine determinations are also important in the food industry [3] and for certain environmental analyses [4]. In this report, an HPLC method with UV detection for the simultaneous detection of nine nitrosamines, two solvents used in the manufacturing processes, and several medications of importance is presented. The nine nitrosamines (NDMA, NMOR, NMEA, NPPYR, NDEA, NPIP, NDPA, NDBA, NDPHA) are separated from other pharmaceutical compounds of interest. Two solvents, DMF and DMA are commonly used in manufacturing processes of sartan drugs. Residual amounts of these solvents are important to detect in both raw processes and final drug products. Therefore, it would be useful to have a reliable protocol for the separation of all nine nitrosamines and the solvents DMF and DMA. As part of this study, two of the columns used were fabricated with a unique type of stationary phase based upon silica hydride as the support material [5,6]. In contrast to ordinary silica, the surface of silica hydride is nonpolar as opposed to polar due to silanols on silica-based stationary phases. This property is used to facilitate the separation of the nitrosamines and the possible presence of solvent residues.

Experimental/Materials

Nitrosamine reference standards were obtained from the Chem Service, Inc (West Chester, PA, USA). Dimtheylformamide (DMF) and dimethylacetamide (DMA) were purchased from Oakwood Products, Inc (Estill, SC, USA). Formic acid (FA) was from EMD (Gibbstown, NJ, USA). Deionised water (DI H_2O) and acetonitrile (ACN) (HPLC grade) were obtained from Sigma-Aldrich, Inc (St. Louis, MO, USA).

Stock solutions of the nitrosamine mixtures were prepared at 1.0 mg/ mL concentrations in a diluent of methanol. A solution of each individual nitrosamine was made at a concentration of 1 mg/mL in order to identify elution order. Drug mixtures were prepared at 1.0 mg/mL concentrations. DMF and DMA solvent solutions were made at a concentration of 10 µL/mL. Losartan and Diovan samples were prepared at a 1.0 mg/mL concentration. Peak abbreviations and chemical names: 1 = NDMA, N-nitrosodimethylamine; 2 = NMOR, N-nitrosomorpholine; 3 = NMEA, N-nitrosomethylethylamine; 4 = NPYR, N-nitrosopyrrolidine; 5 = NDEA, N-nitrosodiethylamine; 6 = NPIP, N-nitrosopiperidine; 7 = NDPA, N-nitrosodi-n-propylamine; 8 = NDBA, N-nitrosodi-n-butylamine; and 9 = NDPHA, N-nitrosodiphenylamine. DMF = dimethylformamide and DMA = dimethylacetamide.

HPLC data was obtained on a Hewlett–Packard (Palo Alto, CA, USA) 1200 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength detector. The system was interfaced with Agilent Chemstation (Santa Clara, CA, USA) software. Method 1 used an analytical column (4.6 mm x 150 mm) packed with a Cogent BDC18™ stationary phase (MicroSolv Technology, Leland, NC). The particle diameter was 4 µm and the pore size was 100 Å. Method 2 employed a 4.6 mm x 150 mm column packed with a Cogent RP C18™ stationary phase. (MicroSolv Technology Corp, Leland, NC, USA). The particle diameter was 3 µm and the pore size was 100 Å. Method 3 used a Cogent UDC column (4.6 x 150 mm) having a 4 µm particle size and a pore diameter of 100 Å. The binary mobile phase solvents for all methods were A: DI water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid, The gradient programs were developed for each column with Method 1 used for the Bidentate C18, Method 2 used for the Cogent RP C18™ and Method 3 for the UDC column. Method 1: Time (min.); %B: 0-1.5; 5: 1.5-15; 60:

15-19; 60: 19-20; 5. Method 2: Time (min.) %B: 0-1.5; 5: 5-15; 70: 15-19; 70: 19- 20; 5. Method 3: Time (min.) %B: 0-5, 5; 5-15, 80; 15-19, 80; 19-20, 5. For all methods the post time was 5 min, the flow rate 1.0 mL/min, the injection volume 1µL and detection in the UV at 220 and 254 nm. The current USP only includes an assay for 7 nitrosamines: NDMA, NDEA, NDIPA, NEIPA, NMBA, NMPA, and NDBA in selected sartans (Diovan and Losartan).

Results and Discussion

Using the Bidentate C18 it was possible to effectively separate Diovan, Losartan and all nine nitrosamine impurities in one method. *(Figure 1A.)* using 254 nm for detection. The BDC18 is also able to resolve the nine nitrosamines from the two potential solvent impurities (Figure 1B Losartan and Diovan show excellent peak shape on the RP C18 column but less resolution from the nitrosamine impurities than the BDC18 *(Figure 2A)*.

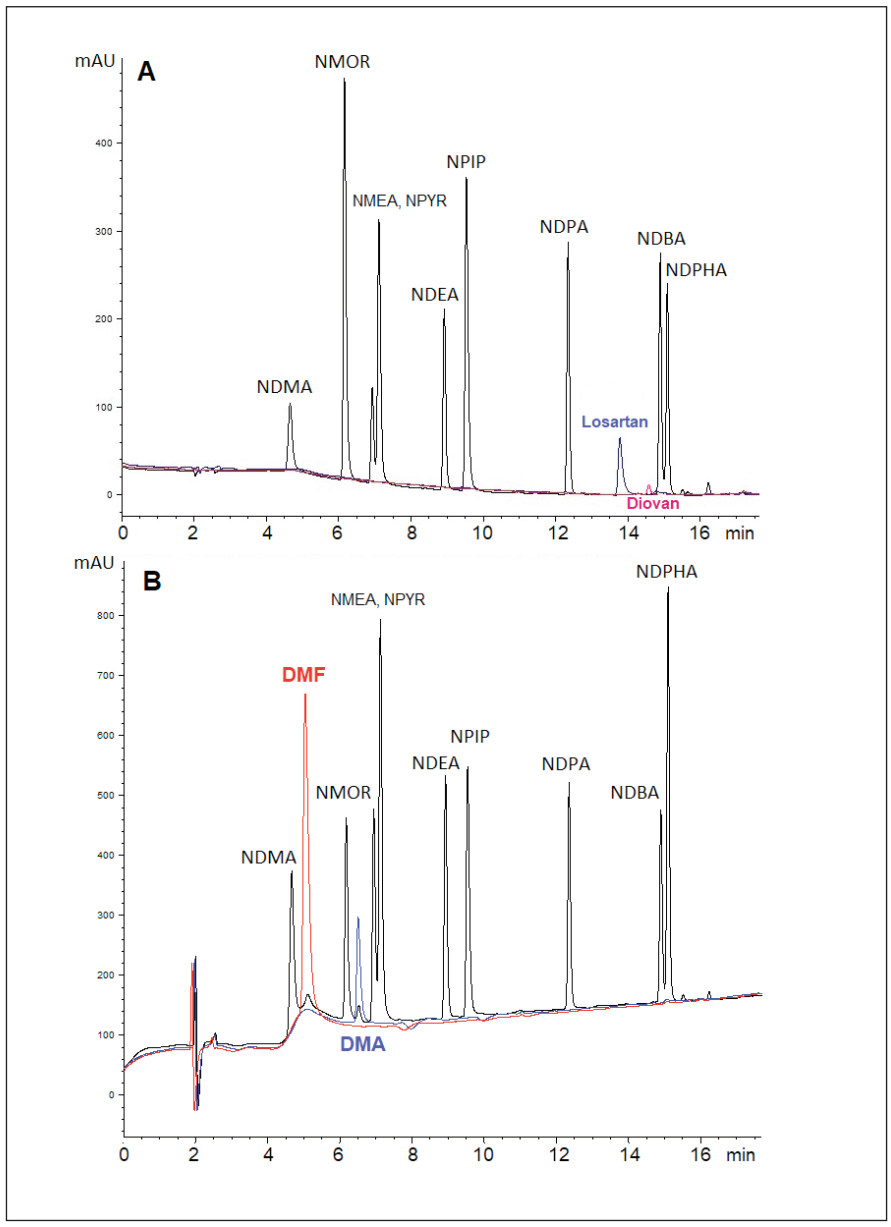


Figure 1. Analysis of the nine nitrosamine standards on the BD C18 column in the presence of A) the drugs Losartan and Diovan and B) the solvents DMF and DMA.

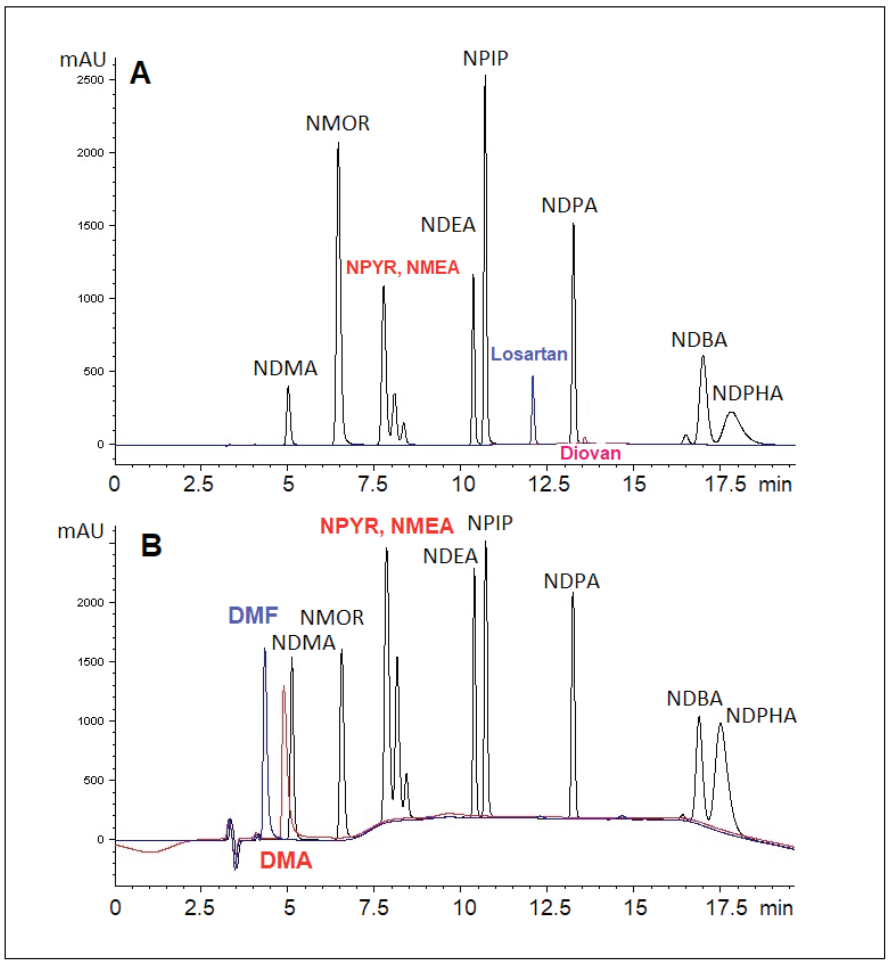


Figure 2. Analysis of the nine nitrosamine standards on the RP C18 column in the presence of A) the drugs Losartan and Diovan and B) the solvents DMF and DMA.

The two sartans are also less retained on the RP C18 in comparison to the Bidentate C18. DMF and DMA are also less retained on the Cogent RP C18 column as well as not completely resolved from one sample component when compared to the BDC18. (Figure 2). It can also be seen that two components, NPYR and NMEA, are reversed in elution order on the two stationary phases. Additional determinations were made on the Cogent UDC stationary phase. The bonded moiety in this column consists of cholesterol with an 11-carbon chain attached to the silica hydride surface. Cholesterol is a liquid crystal as a pure compound and retains some of the ordered structure even when bonded to a chromatographic support surface [6]. Thus, the UDC column possesses in addition to hydrophobic interactions discrimination based on molecular shape. *Figure 3* shows the chromatogram obtained with the optimised gradient. All nine nitrosamine standards are separated as is the case with the two C18 stationary phases. The UDC phase provides much better separation of NPYR and NMEA than either of the octadecyl columns and also show two additional compounds that were present in the NDPH standard and one in the NMEA standard. The differences between the results obtained by the two C18 phases is most likely due to the main distinguishing feature between the two columns, i.e. the particle surface. One is composed of Si-H groups while the other has Si-OH moieties on the surface. It appears that for the purpose of this analysis, the hydride surface on the BD C18 provides somewhat better selectivity than the silanol surface of the RP C18.

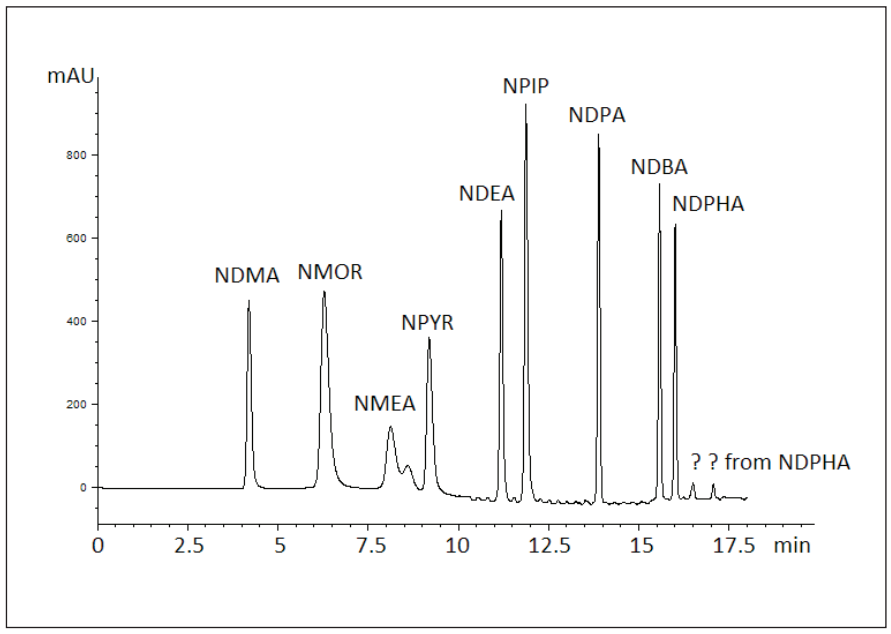


Figure 3. Analysis of the nine nitrosamine standards on the UDC column.

In summary, each method offers separation of both impurities and sartan drugs as well as employing a mass spectrometry-friendly mobile phase. The Bidentate C18 retains and separates all nine nitrosamines and provides adequate resolution from drugs of importance. The Bidentate C18 offers better resolution of DMF and DMA solvent peaks. The Cogent RP C18 can retain and separate these compounds as well but loses peak efficiency of NDBA and NDPHA (nitrosodi-n-butylamine and nitrosodiphenylamine respectively) when compared to the analysis on the Cogent Bidentate C18. Depending on the analyst's needs, the Bidentate C18 method may be better suited for an assay involving a wide range of impurities as well as potential solvent residues whereas the Cogent RP C18 and Cogent UDC may be better for a saratan drug assay with some nitrosamine impurities of interest.

References

1. *M. K. Parr, J. F. Joseph, NDMA impurity in valsartan and other pharmaceutical products: Analytical methods for the determination of N-nitrosamines, J. Pharm. Biomed. Anal. 2019, 164, 536-549.*

2. *S. Schmidtsdorff, A. H. Schmidt, Simultaneous detection of nitrosamines and other sartan-related impurities in active pharmaceutical ingredients by supercritical fluid chromatography, J. Pharm. Biomed. Anal. 2019, 174, 2019.*

3. *Z. Li, J. Wang, X. Chen, S. Hu, T. Gong, Q. Xian, A novel molecularly imprinted polymer-solid phase extraction method coupled with high performance liquid chromatography tandem mass spectrometry for the determination of nitrosamines in water and beverage samples, Food Chem. 2019, 292, 267-274.*

4. *M. H. Chong, M. M. Sanagi, S. Endud , W. A. W. Ibrahim, S. Chien Lau , O. M.L. Alharbi, I. Ali, Determination of N-nitrosamines in water by nano iron-porphyrinated poly(amidoamine) dendrimer MCM-41 generation-3 through solid phase membrane tip extraction and HPLC, Environmental Tech. Innov. 2018, 10, 102-110.*

5. *J.J. Pesek, M.T. Matyska, Silica Hydride Based Packing Materials: HPLC Stationary Phases for a Global Approach to Complex Sample Analysis, Current Chromatography, 2018, 5, 33-42.*

6. *J.J. Pesek, M.T. Matyska, The Development of Silica Hydride Stationary Phases for High-Performance Liquid Chromatography from Conception to Commercialization, Separations, 2019, 6, 27-41.*

Read, Share and Comment on this Article, visit: www.labmate-online.com/article

GC Range Available at Affordable Monthly Payments

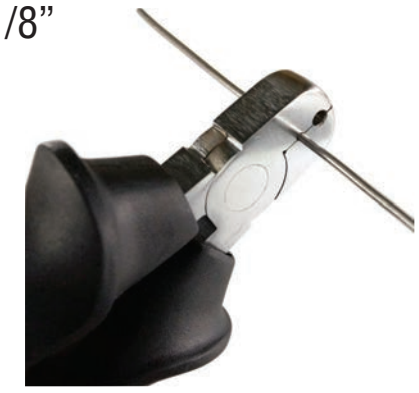
Ellutia Chromatography solutions can now offer a range of monthly payment options for UK customers looking to purchase a GC. Leasing or Hire Purchase means companies do not need to make a large capital outlay and could further benefit from potential tax savings. The already low entry price of the 200 series Gas Chromatograph combined with a range of funding options makes GC Analysis affordable to more laboratories without sacrificing analytical performance. Systems can be configured for manual injection or a range of autosampler options can be added.

The 200 Series Gas Chromatograph from Ellutia is a compact high-performance GC at an affordable price making gas chromatography accessible to every lab. Originally designed for use in education, the 200 Series GC is simple to operate with rugged construction making it the ideal first GC for scientists looking to start Gas Chromatography. The analytical performance however matches much larger costlier instruments from other manufacturers meaning it is just at home in a commercial lab as it is in the classroom.

More information online: ilmt.co/PL/Lq4w

For More Info, email: 56515pr@reply-direct.com

Precision Pliers for 1/16 or 1/8" Stainless Steel HPLC Tubing



Microsolv Technology Corporation's pliers can insure you have good internal flow path in your HPLC tubing to avoid band broadening and other flow issues. Including soft grips and two centre holes where one hole is slightly larger than 1/16" and the other is slightly larger than 1/8". This tool for Chromatography is designed to remove kinks and bends in Stainless Steel tubing or for making seamless bends when you squeeze the Pliers. This will help to insure you have a good internal flow path in your HPLC Stainless Steel tubing and no void problems due to bent or kinked tubing.

The Beta Tool-2™ features a shorter and rounded nose than most precision pliers and it can reach further into an HPLC Instrument to work with tubing that is already installed. You can now enhance your data with improved flow.

More information online: ilmt.co/PL/WQ2Q

For More Info, email: 56945pr@reply-direct.com