COGENT

NITROSAMINE IMPURITY ASSAY BY HPLC

Extended Application Note

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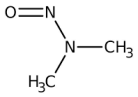

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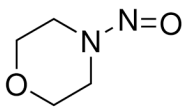

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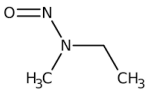

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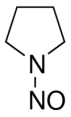

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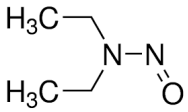
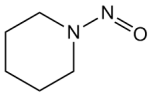
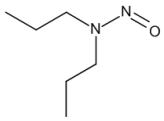


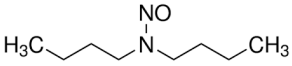
5 Nitrosodiethylamine



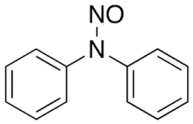
6Nitrosopiperidine



7 Nitrosodi-n-propylamine



8 Nitrosodi-n-butylamine



9 Nitrosodiphenylamine

Figure A

Method 1: Time

(min.)	%B
0	5
1.5	5
15	60
19	60
20	5

Post Time: 5 min Flow Rate: 1.0 mL/min Injection Volume: 1µL Detection: UV: 220/254 nm

Method 2: Time

(min.)	% B
0	5
1.5	5
15	70
19	70
20	5

Post Time: 5 min Flow Rate: 0.5 mL/min Injection Volume: 1µL Detection: UV: 220/254 nm

Introduction

Nitrosamines are highly toxic and are suspected to be a human carcinogen. At high doses, some have 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.

In this extended application note, we present an HPLC method with UV detection for the simultaneous detection of nine nitrosamine impurities, two solvents used in the manufacturing processes, and several medications of importance. The nine nitrosamine impurities (NDMA1, NMor2, NMEA3, NPyr4, NDEA5, NPip6, NDPA7, NDBA8, NDPHA9) are separated from other pharmaceutical compounds of interest.(FigureA) 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. Currently, no published data exists for separation of all nine nitrosamine impurities and solvents: DMF and DMA.

Experimental

Materials

Nitrosamine reference standards were purchased from Chem Service, Inc. (West Chester, PA, USA). Dimethylformamide (DMF) and dimethylacetamide (DMA) were sourced from Oakwood Products, Inc. (Estill, SC, USA). Formic acid (FA) was purchased from EMD (Gibbstown, NJ, USA). Deionized water (DI H2O) and acetonitrile (ACN) (HPLC grade) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

Instrumentation

A Hewlett–Packard (Palo Alto, CA, USA) 1200 HPLC system consisting of an autosampler, degasser, binary pump, and variable wavelength UV detector was used for this analysis. The system was interfaced with Agilent Chemstation (Santa Clara, CA, USA) software. Method 1 analytical column employed a 4.6 mm (i.d.) x 150 mm and packed with a Cogent BDC18™ stationary phase. The particle diameter was 4µm and the pore size was 100Å. Method 2 employed a 4.6 mm (i.d.) x 150 mm and packed with a Cogent RP C18™ stationary phase. (MicroSolv Tech. Corp., Leland, NC, USA). The particle diameter was 3µm and the pore size was 100Å. The binary mobile phase solvents for both methods were A: DI water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid. The gradient programs are shown to the left. Method 1 is used for Bidentate C18™. Method 2 is used for the Cogent RP C18™.

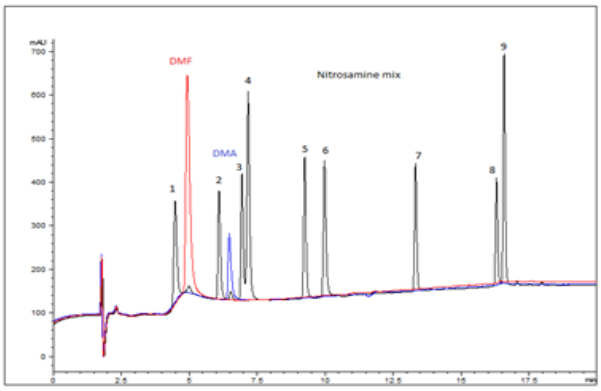


Figure 1.

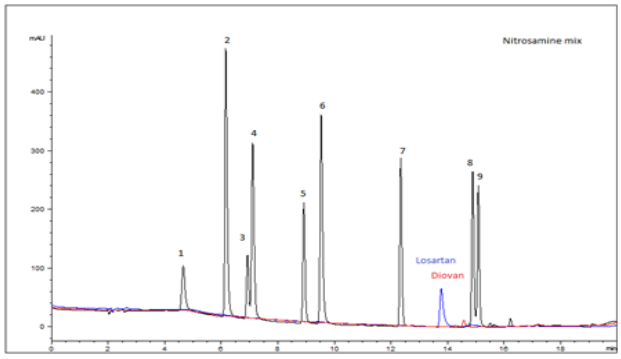


Figure 2.

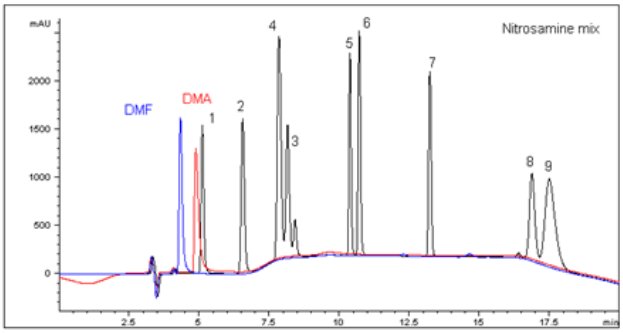


Figure 3.

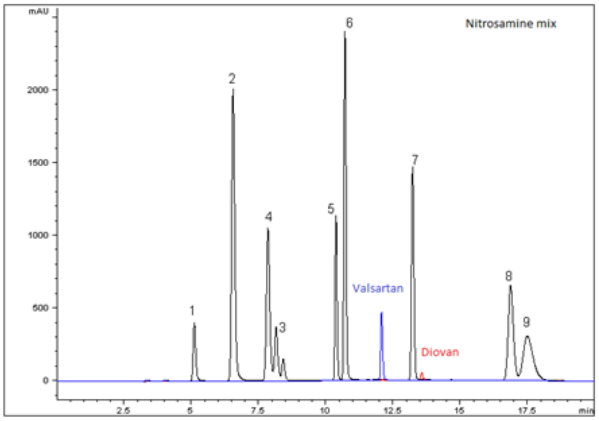


Figure 4.

Sample Preparation

Stock solutions of the nitrosamine mixtures were prepared at 1.0 mg/mL concentrations in a diluent of methanol. Each individual nitrosamine solution was analyzed as a neat solution, provided at a 1 mg/mL concentration, in order to identify elution order. Drug mixtures were prepared at 1.0 mg/mL concentrations. DMF and DMA solvents were prepared at 10 uL/mL concentration. Losartan and Diovan were prepared at a 1.0 mg/mL concentration.

Results and Discussion

The current USP Nitrosamine Impurity Test Method {chapter 1469} includes an assay for 7 nitrosamines: NDMA, NDEA, NDIPA, NEIPA, NMBA, NMPA, and NDBA in selected sartans (valsartan, irbesartan, and losartan potassium).

Using the Bidentate C18 4um 100A 4.6 x 150mm, it was possible to effectively separate DMF, DMA, and all nine nitrosamine impurities in one method. UV detection at 220nm was selected for DMF and DMA (Figure 1.) and 254 nm was selected for nitrosamine impurities. (Figure 2.) The BDC18 also shows resolution of nine nitrosamines and two classes of sartans: Losartan and Diovan.

DMF and DMA are also less retained on the Cogent RP C18 3um 100A, when compared to the BDC18. (Figure 3.) Valsartan and Diovan show excellent peak shape and resolution from impurities in Figure 4.

In summary, each method offers great separation of both impurities and sartan drugs as well as employing a mass spectroscopy-friendly mobile phase. The Bidentate C18™ 4um 100A 4.6 x 150mm retains and separates all nine nitrosamines and provides adequate resolution from drugs of importance. The Bidentate C18™ also offers better resolution of DMF and DMA solvent peaks. The Cogent RP C18™ 3um 100A 4.6 x 150mm can retain and separate these compounds as well but loses peak efficiency of NDBA and NDPhenyl (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 impurities assay whereas the Cogent C18™ may be better for a saratan drug assay with some nitrosamine impurities of interest.

Peak	Abbreviation	Chemical name
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-Nitrosodimethylamine
8	NDBA	N-Nitrosodi-n-butylamine
9	NDPHA	N-Nitrosodiphenylamine