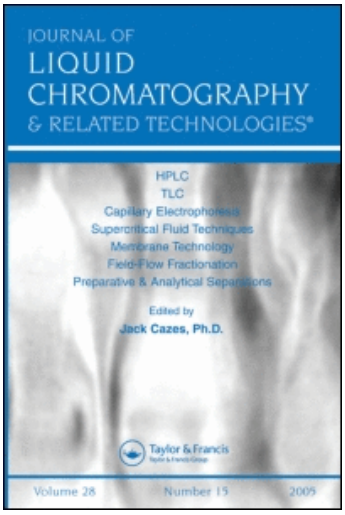

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SIMULTANEOUS DETERMINATION OF m-NITROBENZENESULFONYL CHLORIDE, p-NITROBENZENESULFONYL CHLORIDE, AND THEIR HYDROLYZED ACID PRODUCTS BY NORMAL PHASE HPLC

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ABSTRACT

Meta-nitrobenzenesulfonyl chloride and para-nitrobenzenesulfonyl chloride are reactive and moisture sensitive. A normal phase HPLC method have been developed and validated for the simultaneous determination of the two compounds and their hydrolyzed acid products without derivatization. Fast column re-equilibration and adequate resolution for all compounds of interest are achieved by using a CN column typically used for reversed phase HPLC.

INTRODUCTION

m-Nitrobenzenesulfonyl chloride (m-nosyl chloride) is used as a raw material for the production of ZYVOX, the first of a new class of synthetic antibiotics called oxazolidinones.(1) To ensure the raw material quality, an HPLC assay

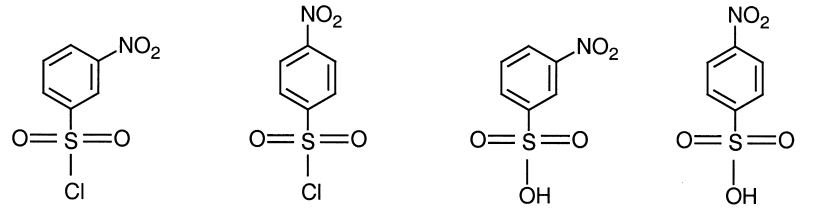

Figure 1. Chemical structures.

method is developed and validated for m-nosyl chloride and its impurities, p-nitrobenzenesulfonyl (p-nosyl chloride), m-nosic acid, and p-nosic acid (Figure 1).

The two sulfonyl chlorides, m-nosyl chloride and p-nosyl chloride, are reactive and sensitive to moisture. Derivatization is usually applied to the sulfonyl chlorides before sample analysis. However, this would increase the amount of sample preparation work, as well as deal with completion of the derivative reaction.

This work demonstrates a novel normal phase HPLC method that could simultaneously determine the compounds of interest without prior derivatization. Further, it used a CN column typically for reversed phase HPLC analysis and achieved fast re-equilibration and adequate resolution for all compounds of interest. The method validation results indicated that this method is suitable for its intended use, raw material release, and quality control.

EXPERIMENTAL

Apparatus

An Agilent 1100 LC system consisting of an automatic degasser system, a quaternary pump, a thermostatic column compartment, an automatic injector system, and a diode array detector (DAD) was used for the method development and validation (Agilent, Palo Alto, CA). Data were collected and processed by Agilent ChemStation. A Mettler-Toledo AT 261 Delta Range® balance with readability of 0.01 mg was used (Mettler-Toledo, Columbus, OH). An Agilent Zorbax® SB-CN Solvent Saver® 3.0x250 mm 5-micron column (Part No. 880975-305) was used.

Materials

All the following reagents were of HPLC grade or analytical grade. Hexanes, isopropanol, and anhydrous tetrahydrofuran were purchased from EM Science (Gibbstown, NJ). Trifluoroacetic acid was purchased from Aldrich (Milwaukee, WI). Water was filtered through the Milli-Q water system and a 0.22 μm Millipak® 40 filter unit (Millipore, Bedford, MA).

The standards of m-nosyl chloride, p-nosyl chloride, and m-sodium nosylate were purchase from Aldrich (Milwaukee, WI).

Chromatographic Conditions

The flow rate was 0.40 mL/min. The injection volume was 2.0 μL. The UV detection was at 250 nm. The run time was 20 min with a 10-min post run for column re-equilibration. The mobile phase was a gradient solvent mixture of hexanes, isopropanol, and water containing 0.5% trifluoroacetic acid. The gradient steps were listed in Table 1.

Standard Preparation

For validation, m-nosyl chloride and p-nosyl chloride were prepared by accurately weighing the standards in 20-mL vials. Fifteen milliliters of anhydrous tetrahydrofuran (THF) was added into the vial by a volumetric pipette. Then, the standards were ultrasonicated for 3 min to complete dissolution. The m-sodium nosylate was used as the standard for m-nosic acid because the UV response of m-sodium nosylate would be the same as that of m-nosic acid in solu-

Table 1. Mobile Phase Gradient Steps

Time, min	Solvent A, %	Solvent B, %	Solvent C, %
0.00	95	5	0
2.00	95	5	0
2.01	30	70	0
3.00	30	70	0
3.01	25	70	5
10.00	20	70	10
10.01	30	70	0
11.00	30	70	0
11.01	95	5	0

tion. Standard m-sodium nosylate was accurately weighed and dissolved in 15 mL 10:90 water:THF (vol:vol) due to its low solubility in THF. Anhydrous THF was used if dilution of standard solutions was needed.

For calibration, the concentrations of standard m-nosyl chloride should be prepared in the range of 500-1,200 μg/mL in anhydrous THF. Relative response factors (RRF) were used for the calibration of impurities, p-nosyl chloride, m-nosic acid, and p-nosic acid. The m-nosic acid was quantified by using response factor (RF) of m-sodium nosylate multiplied by a factor of 0.90 (molecular weight ratio of m-nosic acid and m-sodium nosylate). The p-nosic acid was quantified by using the same RF of the major component m-nosyl chloride because the p-nosic acid standard was not available.

Sample Preparation

Sample solutions were prepared by accurately weighing the samples in 20-mL vials. The weights should be within 10-15 mg. Fifteen milliliters of anhydrous tetrahydrofuran (THF) was added into the vial by a volumetric pipette. Then, the samples were ultrasonicated for 3 min to complete dissolution. THF was used as a sample blank.

RESULTS AND DISCUSSION

Method Development

The m-nosyl chloride and p-nosyl chloride could be baseline resolved easily by using an Eclipse® XDB-phenyl column with 50:50 water:acetonitrile as the mobile phase. Although they are moisture sensitive, both m-nosyl chloride and p-nosyl chloride did not show hydrolysis under this reversed phase condition. However, mobile phase modifiers, e.g., low pH phosphoric buffer, had to be added to retain the acid impurities, m-nosic acid and p-nosic acid. Hydrolysis of m-nosyl chloride was found by observing the rise of the baseline at the tail of the m-nosic acid peak and the front of m-nosyl chloride peak. Therefore, NP-HPLC should be applied for the analysis of these moisture sensitive compounds and their hydrolyzed acids.

A normal phase amine column (NH_2) or a diol column should not be used, because both m-nosyl chloride and p-nosyl chloride are reactive to the amine and alcohol. A CN column was the best choice due to its ability of fast equilibration and its non-reactivity to the sulfonyl chlorides. Alcohol solvents for NP-HPLC such as isopropanol should not be used for sample preparation because the two chlorides are reactive to it. THF was a good solvent for the sample preparation

because of its miscibility to the NP-HPLC solvents and its non-reactivity to the sulfonyl chlorides.

Unfortunately, a normal phase CN column (Zorbax CN column, 4.6x150 mm, 5-μm) did not offer adequate separation of the two chlorides. And low levels of the two acid impurities just could not be detected due to the interaction between the silica-based CN column and the acids.

A Zorbax® SB-CN column (3.0x250 mm, 5 μm), typically used for RP-HPLC, was found to resolve m-nosyl chloride and p-nosyl chloride very well in 5 min by 95:5 hexanes:isopropanol. No degradation of the two chlorides was found, although, they could react to the 5% isopropanol in the mobile phase. The reason might be that 5-min elution time was too short for the reaction to progress significantly to be detected. However, the dissolution of the acid impurities in the 95% hexanes was a problem. To dissolve and resolve the two acids during a run, a small amount of water containing 0.5% trifluoroacetic acid (TFA) was introduced into the mobile phase. Water in the mobile phase helped to dissolve the acids. TFA protonated the acids and helped them being retained and resolved on the column. The SB-CN column is a StableBond HPLC column that provides high column stability at low pH, e.g., pH = 1.8.(2) The silica base of the column is modified to diisopropylsilanes that reduce the interaction between the acid impurities and the column, allowing good resolution and sensitive detection of the two acids.

Both linear gradient and step gradient were used for the separation of the two chlorides and the two acids, simultaneously. These gradient steps would generate system peaks. The gradient steps were carefully monitored to ensure that solvent and system peaks would not interfere with the quantitation of all the four compounds of interest (Figure 2). The critical impurity was p-nosyl chloride. Optimum wavelength for detecting p-nosyl chloride was 250 nm and was used as the UV detection wavelength for this method.

Specificity

Baseline resolution of m-nosyl chloride and p-nosyl chloride was achieved with a resolution value of 2.50. The resolution value of m-nosic acid and p-nosic acid was 1.22. Except for p-nosic acid, the peaks of interest were identified by purchased standards. Identification of p-nosic acid was achieved by the analysis of a hydrolyzed standard p-nosyl chloride solution and by the DAD UV spectrum match.

Linearity

The linear regression coefficients, R(2) were 0.9996, 0.9999, and 1.0000 for m-nosyl chloride of 0.5-1,442 μg/mL, p-nosyl chloride of 0.2-675 μg/mL,

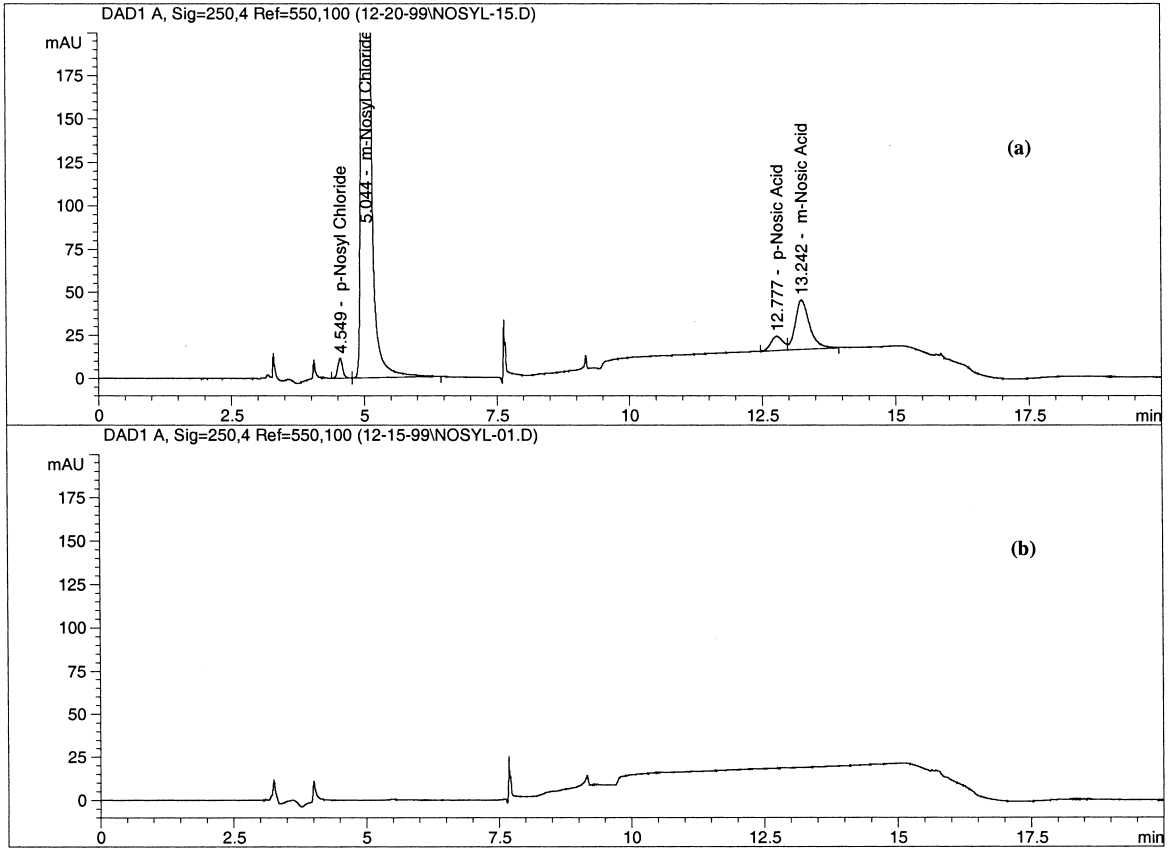

Figure 2. Chromatograms of (a) a standard mixture in anhydrous THF and (b) a blank solvent THF. Chromatographic conditions are described in the Experimental section.

and m-nosic acid of 3.9-492 μg/mL, respectively. The response factor (RF) of a compound of interest was the ratio of the standard concentration in solution to the peak area. The relative response factor (RRF) of a compound of interest was the ratio of the compound's RF to the RF of m-nosyl chloride. The RRF of p-nosic acid was assigned the same as that of m-nosyl chloride because standard p-nosic acid was not available. The relative response factors were 1.00, 0.51, 1.19, and 1.00 for m-nosyl chloride, p-nosyl chloride, m-nosic acid, and p-nosic acid, respectively.

Limit of Detection and Limit of Quantitation

The limits of detection (LOD) for m-nosyl chloride, p-nosyl chloride, and m-nosic acid in the solutions injected were 0.3 μg/mL, 0.1 μg/mL, and 1.7 μg/mL, respectively. The limits of quantitation (LOQ) for m-nosyl chloride, p-nosyl chloride, and m-nosic acid in the solutions injected were 0.5 μg/mL, 0.2 μg/mL, and 3.9 μg/mL, respectively.

Method Precision

Six weights of one m-nosyl chloride sample were assayed with triplicate injections. The average weight % of m-nosyl chloride was 100.2% with a % RSD of 0.9%. In addition, six calibration curves of m-nosyl chloride were prepared for the assay of the m-nosyl chloride sample with 3 weights and duplicate injections. The average weight % of m-nosyl chloride was 100.3% with a % RSD of 0.6%. The average linear regression coefficient, R(2) of the six curves was 0.9991 with a % RSD of 0.1%. The average RF of m-nosyl chloride was 0.1133 with a % RSD of 0.5%.

Accuracy (Recovery)

Known amounts of m-nosyl chloride and p-nosyl chloride were added in the sample solvent, anhydrous THF. Known amounts of m-sodium nosylate were added in the mixed solvent of 10:90 water:THF. Three weights and duplicate injections were applied to each of the three compounds. The average recovery for m-nosyl chloride of 520.2-1162.6 μg/mL was 99.5% with a % RSD of 0.4%. The average recovery for p-nosyl chloride of 3.9-11.8 μg/mL was 108.2% with a % RSD of 2.0%. The average recovery for m-nosic acid of 6.0-18.1 μg/mL was 96.8% with a % RSD of 7.9%.

Table 2. Sample Solution Stability Study

m-Nosyl Chloride			
Conc. µg/mL	Peak Area, 0 Hr	Peak Area, 24 Hrs	% RSD
1021.0	9020.49	8904.88	0.9
204.2	1885.99	1879.19	0.3
2.3	22.49	22.00	1.6
p-Nosyl Chloride			
Conc. µg/mL	Peak Area, 0 Hr	Peak Area, 24 Hrs	% RSD
48.4	910.15	902.62	0.6
5.4	100.30	99.86	0.3
1.1	19.27	18.41	3.2
m-Nosic Acid			
Conc. µg/mL	Peak Area, 0 Hr	Peak Area, 24 Hrs	% RSD
98.4	818.39	815.54	0.2
19.7	145.80	147.46	0.8
3.9	25.63	26.81	3.2

Robustness

Although m-nosyl chloride and p-nosyl chloride are reactive and moisture sensitive, sample solutions were found quite stable in anhydrous THF after 24 hours at ambient temperature. Table 2 lists the comparison of peak areas at time zero and after 24 hours.

A second Zorbax SB-CN column and a second Agilent 1100 LC system were used for the column-to-column and instrument-to-instrument variability study. Sample solutions from the recovery study were analyzed. The % RSD between the two average results obtained by the two columns and two systems were 0.3%, 0.5%, and 6.5% for m-nosyl chloride, p-nosyl chloride, and m-nosic acid, respectively.

CONCLUSIONS

NP-HPLC is the choice for the analysis of the reactive and moisture sensitive compounds of interest. A SB-CN column typically used for RP-HPLC analysis is preferred over other NP-HPLC columns because it is not reactive to the sulfonyl chlorides, fast for re-equilibration, and durable under low pH for the

desired resolution of the sulfonyl chlorides and their hydrolyzed acid impurities. By choosing non-reactive solvents, the sulfonyl chlorides of interest can be analyzed without derivatization. The validation results indicate that the method is sensitive and highly reproducible.

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