

Efficient Separation of Aniline Metabolites Using a SUPELCOSIL ABZ⁺Plus HPLC Column

A novel method for separating aniline metabolites is fast and reproducible. Seven amino phenols and n-oxidized metabolites were separated using a universal, reversed phase SUPELCOSIL ABZ⁺Plus column. There is no need for ion pair reagents in this method and the mobile phase is compatible with mass spectrometry.

Key Words:

- aniline metabolite
- amines
- mass spectrometry

Primary aromatic amines are used in manufacturing processes of pharmaceutical and agricultural chemicals industries. Nearly 1.4 billion tons of the aromatic amine, aniline, was produced in the United States in 1995 (1). Such large quantities of this compound create a need for an efficient and easy method of analysis for aniline and its biological metabolites.

Many of the compounds formed from aniline metabolism in living organisms are more polar derivatives of the original amine, which must be excreted from the organism (2, 3). It is these polar intermediates that may cause cellular damage because of their high intracellular reactivity. The excreted analytes can be acetylated, o-hydroxylated, p-hydroxylated, or N-hydroxylated. To

be effective, an analytical scheme must enable the user to accurately monitor all of these compounds.

A highly sensitive detector is often needed to analyze aniline metabolites because they are formed in nanogram amounts in biological systems. If ultraviolet-visible spectroscopy is used, labeling or tagging of the metabolites becomes necessary. To avoid these added procedures and time, use amperometry and mass spectrometry for detection (4).

In the past, ion pair reagents have been used to increase retention of the more polar aniline metabolites on C18 reversed phase columns. However, ion pair reagents are incompatible with mass spectrometry, and they reportedly degrade the metabolite n-phenylhydroxylamine (5).

The SUPELCOSIL™ ABZ⁺Plus column has unique selectivity and higher polarity than C18 or C8 columns. These characteristics ensure efficient separation of aniline metabolites. The separation was optimized by varying the acetonitrile concentration in the mobile phase, and the temperature. A 25% acetonitrile concentration and a temperature of 40°C provided the best results. This method gave a resolution of 2 between the two closest peaks and a total run time of less than 8 minutes (see Figure A). The effect of a mobile phase gradient was achieved without the need for column re-equilibration or for concern about instrument variations. The method is simple and reproducible.

For comparison, this same method was run with a C18 column (Figure B). The lower polarity C18 column has lower retention than the ABZ⁺Plus column. The primary amine, aniline, exhibits significant tailing due to silanol interactions. To decrease tailing,

Figure A. Aniline Metabolites Resolved Without Using Ion Pair Reagents

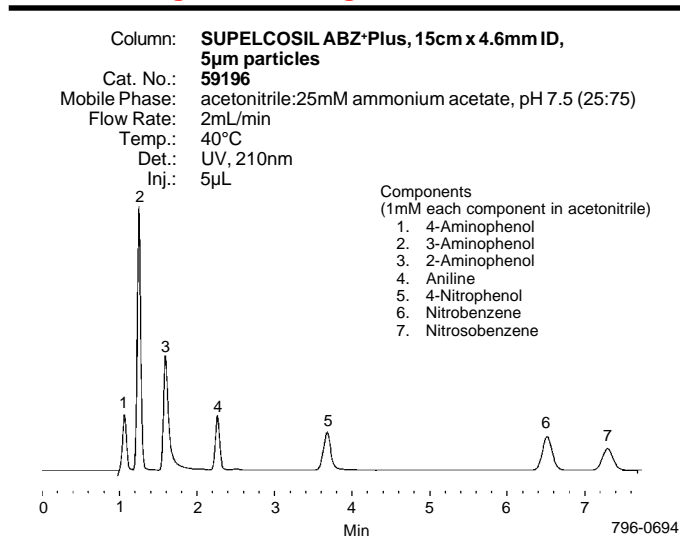
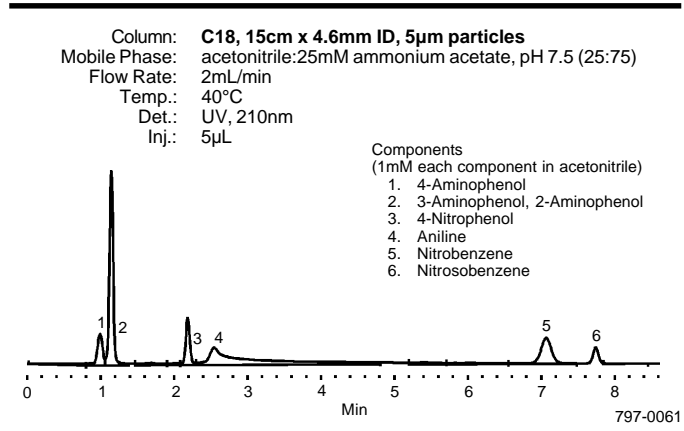


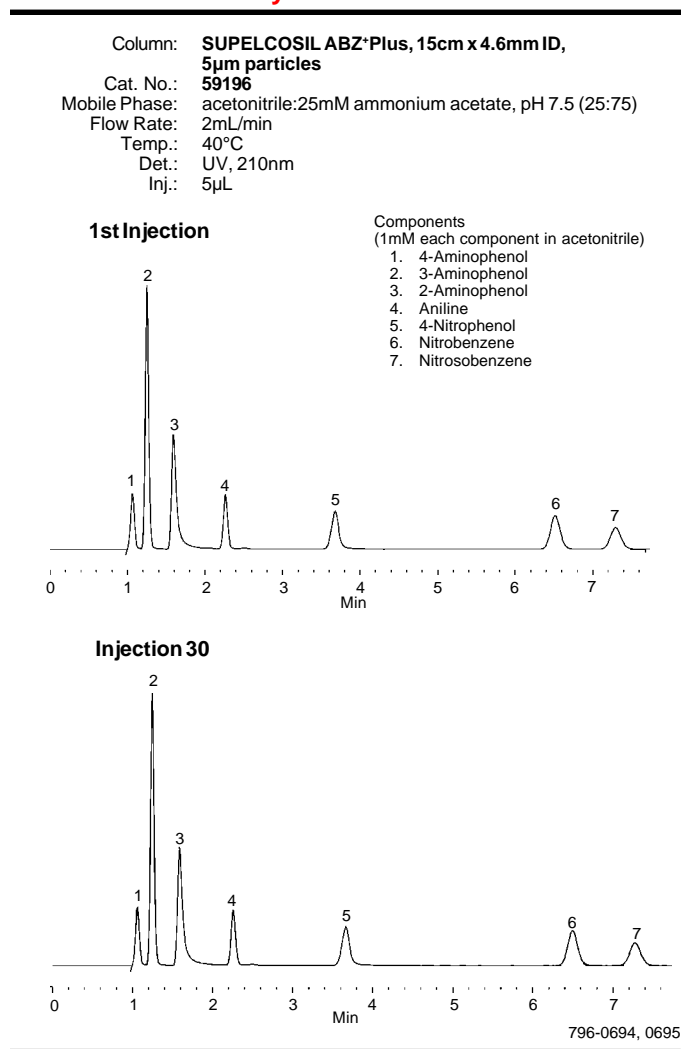
Figure B. Aniline Metabolites on a C18 Column



we would have to use silanol-suppressing components in the mobile phase and a low ionic strength buffer, which would be unsuitable for a mass spectrometer. Therefore, the ABZ⁺Plus column proved to be the only practical choice for separating aniline metabolites.

To confirm the stability and reproducibility of the analysis on ABZ⁺Plus columns, three columns of different bonding and silica lots were tested under the same conditions. Retention times were compared over the course of 30 sequential injections. Figure C shows the first and last injections on one of the ABZ⁺Plus columns. The columns were stable over the test period, with only minor changes in retention times. Efficiency and peak shapes also were unchanged after 30 injections, for all three columns.

Figure C. Aniline Metabolites Resolved Consistently



The coefficient of variation for the retention time of aniline in a column-to-column comparison was 2.28%, and that of nitrosobenzene was 1.92%, indicating that these columns offer a high degree of reproducibility. These low values are well within the specifications needed in many industries. Conventional C18 columns do not perform this reproducibly and efficiently under silanol-suppression conditions.

Analyzing aniline metabolites on an ABZ⁺Plus column is more efficient and reproducible, and provides a better separation, than using a C18 column.

Ordering Information:

| Description | Cat. No. |
|----------------------------------------------------------------------------------|--------------|
| SUPEL COSIL ABZ⁺Plus Column 15cm x 4.6mm ID, 5µm particles* | 59196 |

*Many other dimensions are available. Please refer to our current catalog.

References

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Note 115

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