Application Note: 20444

Determination of Cresols in Urine Samples

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Key Words

- Hypersil GOLD PFP

This application note describes a LC method for the quantitative analysis of cresols in urine using a Hypersil GOLD™ PFP column.

Introduction

Ortho-cresol, para-cresol and phenol are toxic metabolites that are formed in the body after exposure to toluene and benzene. Only after benzene and toluene are metabolized, can ortho-cresol, para-cresol and phenol be excreted[1]. Determination of the concentration of cresols in urine is therefore an important tool for the biological monitoring of toluene and benzene exposure at work.

Benzene is a toxic substance, used as industrial solvent and precursor in the production of drugs, plastics and dyes. It is a human carcinogen and is linked to increased incidence of leukemia in humans. Toluene is a harmful substance widely used as solvent. It is a central nervous system depressant; toluene attacks the liver and kidney and causes cardiac arrhythmia[2].

For any hazardous substance or its metabolites, it is very important to define a threshold value for maximum exposure, which assures safe working conditions. Although there are direct tests to determine exposure to benzene (e.g., benzene in the breath or in the blood), measurements are accurate only for recent exposures. For a more reliable and accurate way to monitor toluene and benzene exposure the analysis of cresols is the main tool. It is therefore essential to have sensitive analytical methods in place to allow detection and quantitation of traces of cresols.

The method described in this application note allows the reliable and fast chromatographic determination of ortho- and para-cresol as well as phenol in urine under isocratic HPLC conditions. This method employs an optimized chromatographic separation with UV detection. The inclusion of an internal standard in the method assures high precision and confidence in quantifying the analytes.

Materials and Method

Column: Hypersil GOLD PFP 5 µm, 150 x 4.6 mm
Part Number: 25405-154630
Mobile Phase: A – H_2O (20%)
B – MeOH (80%)
Flow Rate: 1.3 mL/min
Temperature: 25 °C
Detection: UV at 215 nm
Injection Volume: 10 µL
Ortho-cresol, para-cresol, phenol and benzyl alcohol stock solution concentrations: 0.1 mg/mL.

Results

Hypersil GOLD PFP columns utilize a perfluorinated phenyl stationary phase. The presence of the fluorine atoms on the phenyl ring causes changes in solute-stationary phase interaction. These changes are particularly significant for aromatic compounds where pi-pi interactions are increased, leading to changes in selectivity and extra retention. In this study, Hypersil GOLD PFP was found to offer the selectivity necessary to separate the three polar analytes and the internal standard, as shown in Figure 1.

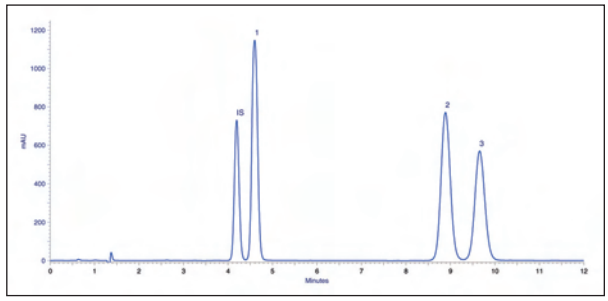

Figure 1: Chromatography for internal standard (IS), phenol (1), p-cresol (2), and o-cresol (3)

To demonstrate the suitability of this method for quantitative analysis, a linearity study was carried out. The linearity of response was determined in the 0.1-100 µg/mL range for each analyte. The results of this study are reported in Figure 2. The following R^2 values were determined: Phenol = 0.98; p-cresol = 0.99; o-cresol = 0.99. Inter-run reproducibility analysis gave the results reported in Table 1.

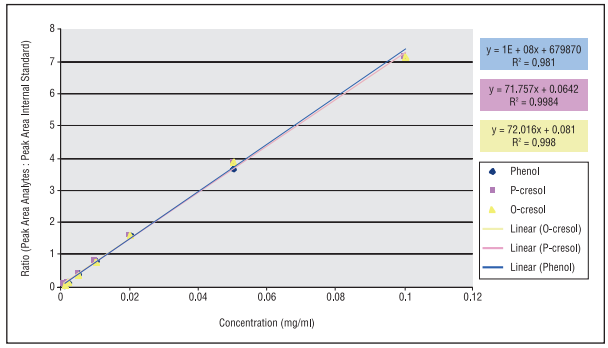

Figure 2: Linearity study for phenol, p-cresol and o-cresol.

	phenol	p-cresol	o-cresol
Mean Response Factor	1.63	1.51	1.52
Standard Deviation	0.00	0.01	0.01
RSD (%)	0.29	0.39	0.61

Table 1: Inter-run reproducibility data

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Conclusions

An isocratic HPLC method has been developed for the determination of phenol, ortho- and para-cresol in urine. The use of benzyl alcohol as internal standard allowed quantitation of the three metabolites, with a linear range comprised between 0.1-100 µg/mL. Good inter-run reproducibility was also achieved.

References

1. Nakajima, T, Wang RS, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H, Pelkonen O, Vainio H, Aoyama T; Biochemical Pharmacology 53 (3): 271-7.
2. NIOSH, National Institute for Occupational Safety and Health, 2005-151.

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