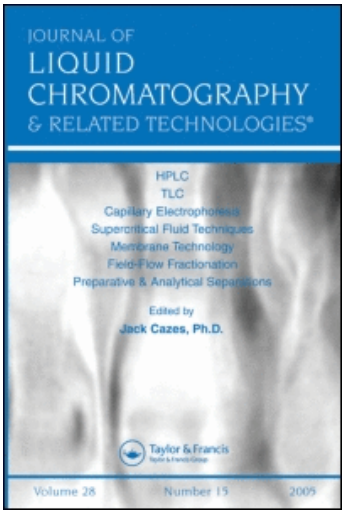


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# Determination of p-Cresidine Sulfonic Acid and p-Cresidine in p-Cresidine Sulfonic Acid for Industrial Use by Reversed-Phase Ion-Pair HPLC

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**Abstract:** A reversed-phase ion-pair high performance liquid chromatographic method was developed to determine p-cresidine sulfonic acid and p-cresidine. Separation was accomplished with a Kromasil $C_{18}$ column with methanol-tetrabutylammonium chloride (10 mM)-water (40/43/17, V/V/V) as mobile phase; detection was performed at a wavelength of 287 nm by UV absorption. The application of the method provides a rapid and efficient technique for the quantitative determination of p-cresidine sulfonic acid and p-cresidine in the industrial processing of p-cresidine sulfonic acid.

**Keywords:** p-Cresidine sulfonic acid, p-Cresidine, Ion-pair, High performance liquid chromatography

## INTRODUCTION

p-Cresidine sulfonic acid (4-amino-5-methoxy-2-methylbenzenesulfonic acid, p-CSA) is an intermediate that is used to make azo, organic, or food dyes.[1,2] p-CSA is commonly obtained by sulphonation of p-cresidine (2-methoxy-5-methylaniline),[3] so there is some trace reactant, p-cresidine,

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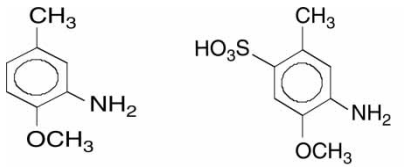
in the final product. p-Cresidine is reasonably anticipated to be a human carcinogen, based on sufficient evidence of carcinogenicity in experimental animals.[4] Contacting p-cresidine can irritate the skin and eyes. Breathing p-cresidine can irritate the nose and throat. High levels can interfere with the ability of the blood to carry oxygen, causing headache, fatigue, dizziness, and a blue color to the skin and lips (methemoglobinemia). Higher levels can cause trouble breathing, collapse, and even death. It is very important to control p-cresidine residues in p-CSA products. Diazotization titration is a classical method for the assay of p-CSA, but the operation's steps are tedious and time-consuming; also, this procedure cannot distinguish p-CSA and p-cresidine. The methods developed previously for p-cresidine analysis include high performance liquid chromatography (HPLC)[5,6] and gas chromatography (GC),[7] but no protocol has been reported for simultaneous determination of p-CSA and p-cresidine. In this paper, a reversed-phase ion-pair HPLC method was applied for this purpose, and the results obtained were satisfactory.




p-Cresidine p-Cresidine sulfonic acid



## EXPERIMENTAL

### Apparatus and Chromatographic Conditions

Instrumentation included a Varian 5060 liquid chromatograph (Varian, Walnut Creek, California, USA), a Rheodyne 7725i injector valve equipped with a 10-μL loop (Rheodyne, Cotati, California, USA), and a Waters 486 tunable UV absorbance detector (Waters, Milford, Mass, USA). The chromatograms were recorded on a Yokogawa Hokushin Electric Type 3066 pen recorder (Sino-Japanese Sichuan Fourth Meter Factory, Chongqing, PRC), and a model JS-3030 chromatographic working station (Dalian Johnsson Separation Science and Technology Corporation, Dalian, PRC). A Waters Alliance 2695 Separations Module, equipped with a vacuum degasser, a quaternary pump, an auto-sampler, and a 996 UV-Vis photodiode-array detector (PDA) (Waters) was used for collection of PDA spectra of the components determined.

The column was a Kromasil $C_{18}$, 150 mm × 4.6 mm I.D., packed with 5 μm particles (Hanbang Science and Technology Co. Ltd. Jiangsu, Huaian, PRC). The mobile phase was methanol-tetrabutylammonium chloride (TBACl, 10 mM)-water (40/43/17, V/V/V). The separation was carried

out by isocratic elution with a flow rate of 1.0 mL/min, and the column temperature was maintained constant at 30°C. The UV detector was set at 287 nm. The quantitation was based on the calibration curve prepared by peak area measurement.

## Reagents and Chemicals

Reference samples of p-cresidine sulfonic acid and p-cresidine were provided by China National Chemical Construction Jiangsu Company (Nanjing, PRC). Methanol was HPLC grade (Hanbang). Water used for all solutions, dilution, and mobile phase was distilled twice from quartz. Tetrabutylammonium chloride and sodium hydroxide were of analytical-reagent grade.



## Procedure

### Preparation of Solvent A

Because p-CSA only dissolved in an alkaline medium, solvent A was prepared for all standard and sample solutions with the ratio of methanol-sodium hydroxide (1%)-water (40/43/17, V/V/V) similar to the mobile phase proportions.

### Calibration Curves

p-CSA and p-cresidine standard solutions used for calibration purposes were, respectively, prepared by accurately weighing about 25 mg of p-CSA RS and p-cresidine RS into a 25-mL volumetric flask and adding solvent A to make up to the mark. The stock concentrations of p-CSA and p-cresidine are 1.0 mg/mL. Standard solutions, at concentration of $0.0001 \sim 0.8$ mg/mL of p-CSA and $0.0001 \sim 0.1$ mg/mL of p-cresidine, were prepared by serial dilution of the stock solution with solvent A.

### Analysis of Main Content in the Sample

The sample solution was prepared by accurately weighing about 10 mg of p-CSA sample into a 10-mL volumetric flask and adding solvent A to make up to the mark. Transfer 1.00 mL of the obtained solution into a 10-mL volumetric flask, then complete to volume and mix. Ten microliters of the sample solution was injected into the column.

Analysis of Impurities in Sample

The method dealing with samples was the same as described above, but a dilution approach was not included in this process. Ten microliters of the sample solution was injected into the column.

## RESULTS AND DISCUSSION

### Chromatogram

A chromatogram demonstrating the separation of a mixture of p-CSA and p-cresidine is shown in Figure 1. As can be seen, the order of the retention time is: impurity 1 (unidentified), p-CSA, and then p-cresidine, with retention times of 3.0 min, 3.6 min, and 11.6 min, respectively.



### Linear Range and Detection Limit

A regression analysis of the relationship between peak areas versus amounts of standard was carried out over the concentration range studied in a 10 μL injection volume. The linear equations were $A_{p\text{-}CSA}$ (area) = 6937.74381 + 7.98117 × $10^7 C_{p\text{-}CSA}$ and $A_{p\text{-}cresidine}$ (area) = −60016.43095 + 1.19419 × $10^8 C_{p\text{-}Cresidine,}$ respectively, with correlation coefficients of 0.9999 and 0.9996. The relative standard deviations (RSD) of peak areas for five

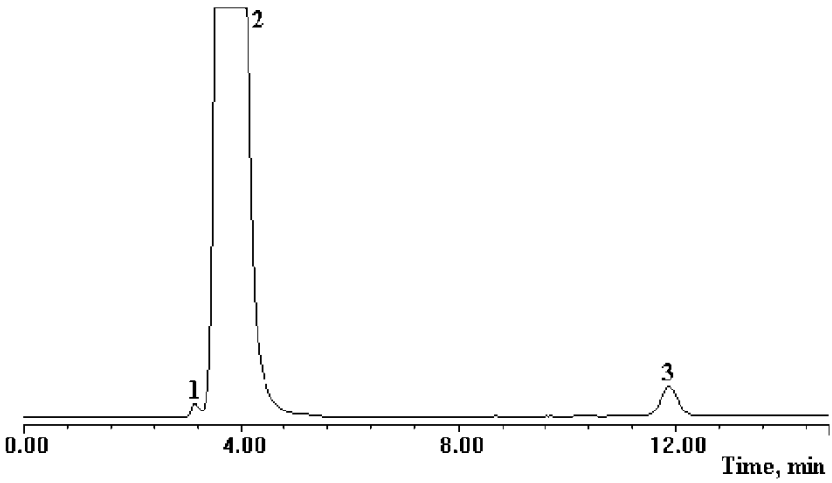



***Figure 1.*** Chromatogram of mixed solution. Peaks: 1. Impurity 1; 2. p-CSA; 3. p-cresidine.

replicate measurements of 1.0 mg/mL p-CSA and 0.006 mg/mL p-cresidine were 0.84% and 0.91%, respectively. The limits of detection (S/N = 3) were 0.0001 mg/mL for p-CSA and p-cresidine.

## Results for Sample Analysis

One sample of p-CSA industrial product was analyzed for p-CSA and p-cresidine contents under the optimum experimental conditions (Figures 2 and 3). The results are summarized in Table 1. The result for p-CSA by diazotization titration is higher than that by the proposed HPLC method, due to total content of the components containing amino-groups. In order to estimate the efficiency of the recovery, the sample was spiked with these two components of interest. Table 2 shows the concentrations of p-CSA and p-cresidine added, and the percentage recoveries.



## Choice of Experimental Parameters

### Mobile Phase

In the aqueous mobile phase, p-CSA dissociated into hydrophilic sulphonate anion and, therefore, is not retained on the $C_{18}$ stationary phase. The ion-pair approach was, therefore, thought of as an alternative in order to enhance retention and improve the separation. Ion-pair reagent TBACl was added into the mobile phase to form a hydrophobic compound, combining

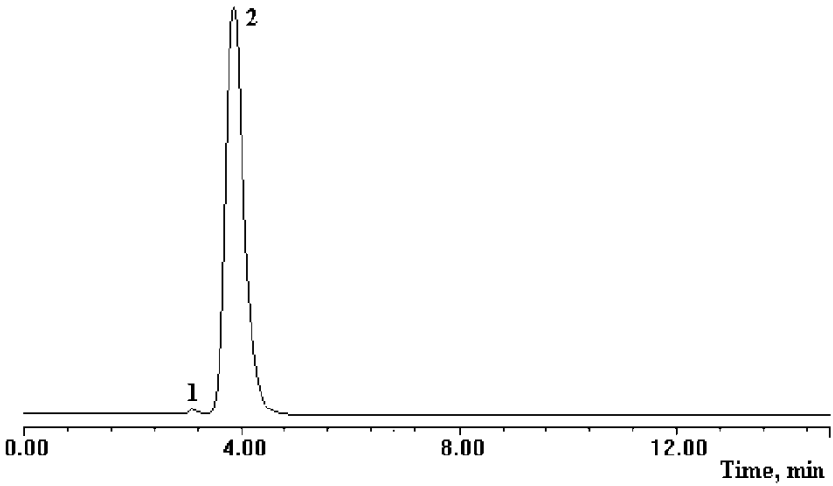



*Figure 2.* Chromatogram of main content in p-CSA sample. Peaks are as in Figure 1.

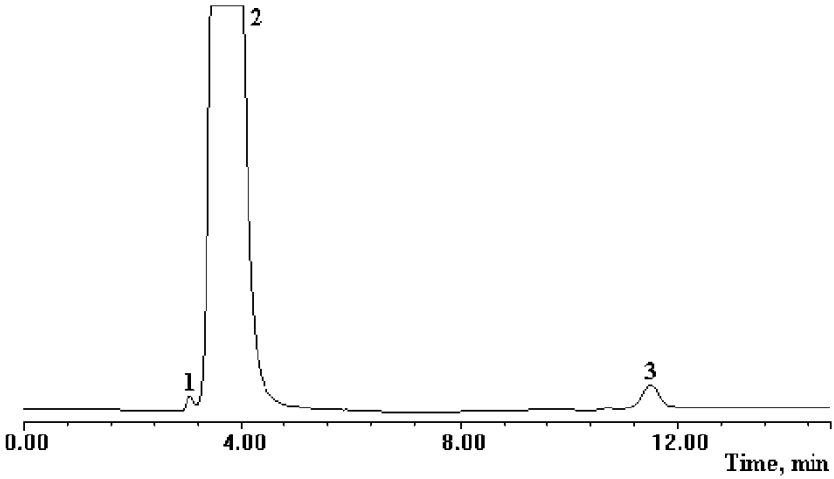



*Figure 3.* Chromatogram of impurities in p-CSA sample. Peaks are as in Figure 1.



quaternary ammonium cation with the sulphonate anion. Various methanol ratios and TBACl concentrations were adjusted for optimum separation conditions.

The results of the test indicated that the p-CSA peak eluted near the solvent peak using mobile phase without TBACl. Retention time wasn't prolonged with the decrease of methanol proportion, but increased with the increase in TBACl concentration (Figure 4). A balance was achieved at 3.0 mM TBACl in the mobile phase. However, for this ion-pair reagent concentration, i.e., below 3.5 mM, the p-CSA peak was covered with the impurity 1 peak, and also for the TBACl concentration above 4.4 mM, the chromatogram of p-cresidine was characterised by a broad asymmetrical peak which is unsuitable for quantitative analysis. Thus, the optimal range of TBACl concentration was selected as 3.5–4.4 mM.

It is shown, from the experiments, that retention behavior of impurity 1 resembled p-CSA and wasn't separated from p-CSA without TBACl in the mobile phase. Therefore, impurity 1 was speculated to be an isomer of p-CSA or multi-sulphonation product.

***Table 1.*** Precisions of analyses (n = 6)

| | Within-day | | Between-day | | Diazotization |
|---|---|---|---|---|---|
| Component | g/100g | RSD (%) | g/100g | RSD (%) | (g/100g) |
| p-CSA | 86.30 | 0.59 | 86.28 | 0.69 | 86.75 |
| p-cresidine | 0.412 | 0.84 | 0.399 | 2.73 | |

*Table 2.* Recoveries of p-cresidine sulfonic acid and p-cresidine (n = 3)

| | Concentration (mg/mL) | | | |
|---|---|---|---|---|
| Component | Initial | Added | Determined[a] | Recovery (%) |
| p-CSA | 0.0847 | 0.0426 | 0.1264 (1.12) | 97.9 |
| | | 0.0852 | 0.1689 (0.10) | 98.8 |
| | | 0.1704 | 0.2591 (0.36) | 102.3 |
| p-cresidine | 0.0038 | 0.0012 | 0.0050 (1.46) | 100.0 |
| | | 0.0025 | 0.0064 (3.51) | 104.0 |
| | | 0.0050 | 0.0089 (0.89) | 102.0 |

[a]Data in bracket are RSD (%).



Neither too high nor too low methanol ratio adapted to separation resulted by comparing different methanol ratios with the same concentration of TBACl (4.3 mM). Thereby, 40% methanol was selected. In the test of chromatographic conditions, buffer with pH value 7.0 was added into mobile phase to improve the peak shape and symmetry. The retention time of p-CSA was close to the dead time and overlapped with impurity 1 peak with mobile phase containing this buffer, but no TBACl. While using $NH_3$-$NH_4Ac$

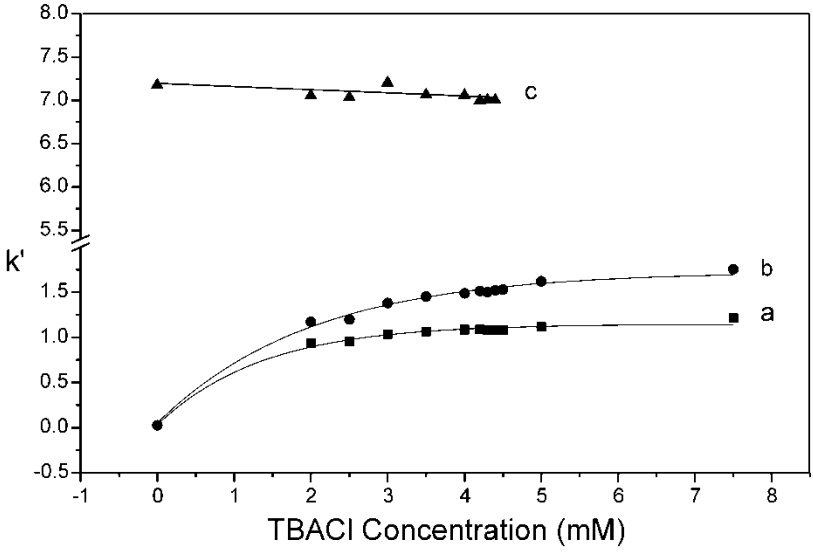



*Figure 4.* Effect of ion-pair reagent concentration upon the capacity factor of (a) impurity 1, (b) p-CSA, and (c) p-cresidine.

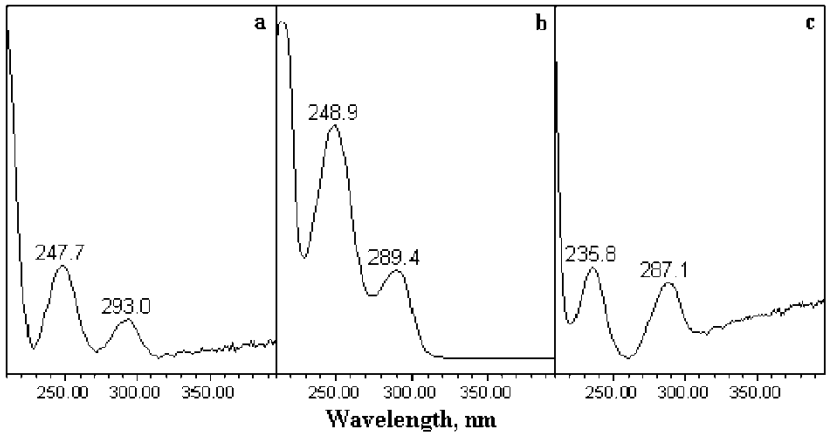



***Figure 5.*** UV absorption spectra for (a) impurity 1, (b) p-CSA, and (c) p-cresidine.



buffer and TBACl, the shape of p-CSA peak wasn't better and sometimes multiple peaks appeared. So the buffer was not employed.

Detection Wavelength

Through the PDA scanning range of 200–400 nm, the strongest absorption peak was found to be near 290 nm for p-CSA and p-cresidine (Figure 5). Selecting 287 nm as the detection wavelength insured analysis sensitivity for both components, especially for the latter. Moreover, impurity 1 exhibited a similar UV absorption spectrum to that of p-CSA, which offered more powerful proof for the estimation mentioned above for the impurity 1.

Sample Concentration

Considering determination of all substances in the sample, the low sample concentration was optimized to determine the major component, whereas the higher was used to determine impurities. If sample concentration was too dense for the main peak to fall into the linear range while determining p-CSA content, the quantitation was incorrect. Too low a sample concentration would lead to disappearance of the impurity peak. Experimental precision looked reasonable after 10X dilution.

## CONCLUSIONS

In the present study, the contents of p-CSA and p-cresidine can be simultaneously determined accurately by reversed-phase ion-pair liquid

chromatography. The method is suitable for routine analysis for production processes and quality control of p-CSA, since it is easy to perform and is quantitative and reproducible.

## ACKNOWLEDGMENT

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