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Separation of phenols as neutral compounds by micellar electrokinetic capillary chromatography

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Abstract

Separation of phenols as neutral solutes by micellar electrokinetic capillary chromatography provides a quantitative linear dynamic range of 6000–13 000. Since the compounds are injected and separated as neutral solutes, the dispersive processes of anti-stacking and electrodispersion are eliminated. Optimized conditions allow for sub-ppm quantitation of trace impurities in the presence of the major components at various stages of the production of high purity phenols. The background electrolyte consists of 100 m*M* sodium dodecyl sulfate in 50 m*M* phosphate buffer pH 7. The method is precise, reliable, and the limits of detection are superior compared to HPLC by a factor of 20.

Keywords: Phenols

1. Introduction

Dakota Gasification Company (DGC) manufactures 15×10^6 kg/year of high quality phenol and cresylic acid, a mixture of cresol isomers. Quality control for 99.95% phenol is quite rigorous as trace analysis for numerous minor impurities is required. The determination of these impurities in phenol process streams is performed at DGC by an internally developed HPLC method. The usual deficiencies with HPLC are slow speed and high solvent consumption but most importantly, closely eluting impurities may be obscured by the major component. This is particularly troublesome when limits of detection (LODs) in the low ppm range are required.

The high resolving power of CE is often advantageous when separating closely related solutes. A significant problem with impurity determinations by CE arises from bandbroadening caused by the charged major component. That component may exhibit electromigration dispersion and its high concentration may cause anti-stacking of the related impurities. This limits the concentration of the major component to approximately 1 mg/ml. At this sample concentration, detection of trace impurities is sometimes difficult.

There are several approaches to be considered for measuring trace components by CE. It may be possible to use sample preparation to remove the major component but this approach was never seriously considered. Sample self-stacking [1], a form of transient isotachophoresis was also considered. The advantage here is that only the trace components are

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enriched. Since this CE system was required for around-the-clock separations by laboratory technicians, this method needed to be simple and rugged.

If all of the analytes, particularly the major component, could be separated as neutral solutes, the problems of electromigration dispersion and anti-stacking would be eliminated. In this case, higher concentration solutions could be prepared compared to the situation where the solutes are charged.

Neutral compounds do not separate by electrophoresis unless secondary equilibrium is employed. This entails the use of charged surfactants and/or cyclodextrins. The determination of phenols and related compounds as neutral solutes by micellar electrokinetic capillary chromatography (MECC) is not new. References are found from the early days of MECC [2,3]. In fact, MECC was designed for the separation of neutral components. What is new and novel is the use of solute neutrality to expand the linear dynamic range of quantitative analysis. In this paper, we report the analytical figures of merit for a variety of phenolic compounds in several sample matrices from industrial synthetic process streams.

2. Experimental

2.1. Reagents

Standard solutions of phenol, catechol, 3- and 4-methylcatechol and *o*-, *m*- and *p*-cresols were purchased from Absolute Standards (Hamden, CT, USA). The CE reagents consisted of 50 m*M* phosphate buffer, pH 7 from Agilent Technologies (Wilmington, DE, USA), and sodium dodecyl sulfate (Ultra) was from Sigma (St. Louis, MO, USA). All of the real samples were 1% aqueous solutions from Dakota Gasification (Beulah, ND, USA). For HPLC, LC-grade acetonitrile, 1% acetic acid and Milli-Q water were employed.

2.2. Instrumental

An Agilent CE system was used for feasibility studies and proof of principle. A 72-cm (length to detector)×50 μm I.D. capillary was used. The capillary was flushed with 1 *M* sodium hydroxide prior to first use. In-between runs, the capillary was flushed with the background electrolyte (BGE). The BGE was 50 m*M* SDS, 50 m*M* phosphate buffer, pH 7. The capillary temperature was 25 °C. The voltage was 20 kV. Injection was 1000 mbar s and detection was UV, 195 nm.

The method was validated on a Beckman (Fullerton, CA, USA) PACE MDQ system. The experimental conditions differed somewhat from the feasibility studies. The capillary was 57 cm (length to detector)×75 μm I.D. The SDS was increased to 100 m*M*. The temperature was 40 °C. Injection was 2.5 p.s.i. s and detection was UV, 200 or 214 nm (i p.s.i.=6894.76 Pa).

Beckman modular liquid chromatographs were used for all separations. The column was a LichoCART 250-4. 250×4.0 mm packed with 3 μm ODS Superspher 100 particles. For HPLC of cresols in high-purity phenol product, the mobile phase was acetonitrile–1% aqueous acetic acid (30:70, v/v), 1%. After the run was completed (16 min), the mobile phase was adjusted to 100% acetonitrile over 2 min, held for 18 min, ramped to the initial conditions over 2 min and held for 6 min prior to beginning the next run. The total run time was 44 min. The detector was set to 275 nm. Fixed-loop injections of 100–200 μl were employed. For LC of phenols and catechols in cresylic acid, the mobile phase contained 35% acetonitrile. The gradient program was identical to phenol.

2.3. Sample preparation

For HPLC, dissolve a 1–4-g sample (determined by concentration of analyte of interest) in acetonitrile to a volume of 10 ml. Dilute the sample 10× further with 25% acetonitrile containing an appropriate concentration (usually 200–500 ppm) of the internal standard, 3,4-xylenol.

For MECC prepare a 1% solution of sample using BGE containing an appropriate concentration of resorcinol as the internal standard.

The linear dynamic range was assessed by spiking phenol samples with the appropriate levels of impurities. The impurity levels were increased until the onset of significant bandbroadening.

3. Results and discussion

3.1. Impact of pH on the separation

Fig. 1 shows separations of phenol and the three cresol isomers by MECC using 50 m*M* SDS and 50 m*M* phosphate buffer, adjusted with solid sodium hydroxide to several pHs. The pK_a of phenol is 10. At pH 11 the net charge on phenol is 0.9. A broadened peak for phenol with the characteristic saw-tooth peak shape indicative of electrodispersion is found. There is no separation of the cresols. At pH 10, the peak shape of phenol improves but the cresol separation remains absent. At pH 9, sharp peaks are found for the cresols that appear to have some isotachophoretic component. At pH 7, good peak shapes are found for all solutes and the cresol isomers are resolved. At that pH, 3 units below the pK_a of phenol, the solutes are for the most part completely protonated.

The pH at the surface of an SDS micelle can be much lower compared to the bulk solution. This is because the electrical double layer at the micellar surface is largely composed of protons. Because a weak acid is being determined, the surface pH effect is unimportant. If a base is being neutralized, it may be necessary to employ a cationic surfactant that has an anionic double layer to ensure solute neutrality. It may not be possible in some cases to ensure that all components in a mixture are indeed neutral. It is only critical that the major components be neutral. Trace components never exhibit electrodispersion and their concentration will be too low to effect any significant anti-stacking.

Fig. 2 shows electropherograms of a 1% solution of cresylic acid. Note the scale setting of the *y*-axis, about 2 absorbance units full scale (AUFS). There is some peak broadening of the cresols but much less than would be expected had the solutes been charged. The peak shape is symmetrical and not saw-tooth as would be expected for electromigration dispersion. This bandbroadening is likely due to saturation of the micellar phase. The electropherogram at the bottom of the figure is expanded to about 130 AUFS. Note that the low concentration phenol and cresol peaks are perfectly symmetrical. In other words, the separation is free from anti-stacking.

Electropherograms from some other portions of the process streams are shown in Fig. 3. For the sample from the dehydration column overheads, all peaks are sharp and symmetrical despite a signal height of 1.2 AU for catechol. The sample from the acid flash overheads shows some broadening of phenol at a signal height of 2.3 AU. This sample should be further diluted by a factor of 2–3.

3.2. Analytical figures of merit

The LODs and limits of quantitation (LOQs) for phenolic impurities are given in Table 1. All of these data met our required needs for detection of impurities.

Table 2 contains data showing the linear dynamic range of this method. The upper limit was defined by the onset of bandbroadening or loss of resolution. The dynamic range is high by HPCE standards and the full range of detector linearity is employed.

The repeatability of the method is given in Table 3. The migration time RSDs were determined using five runs on each of 2 days and was better than 1% except for the cresols. The peak area ratio RSDs ranged from 0.28 to 2.58%. The data from day 2, run several months after day 1 was superior. This was probably the result of increased operator experience. HPLC retention time RSDs ranged from 0.36 to 0.61%, whereas the peak area RSDs were from 0.2 to 3.8% (average=1.9%).

3.3. Comparison with HPLC

Fig. 4 shows an electropherogram of phenol spiked with 5 ppm of each cresol isomer. Fig. 5 illustrates a chromatogram of phenol spiked with 200 ppm of each cresol. By HPLC, *o*- and *m*-cresols coelute and all three isomers elute on the tail of the phenol. As a result, the LODs are 20× poorer compared to capillary electrophoresis. Figs. 6 gives an electropherograms of cresylic acid spiked with 20 ppm of catechol, phenol, and 3- and 4-methylcatechol.

These are four separate HPLC methods that are used at DGC for phenol process monitoring. While the chromatographic run times are comparable to that of electrophoresis, the overall cycle time is at least 3× longer by LC because of the need for extensive

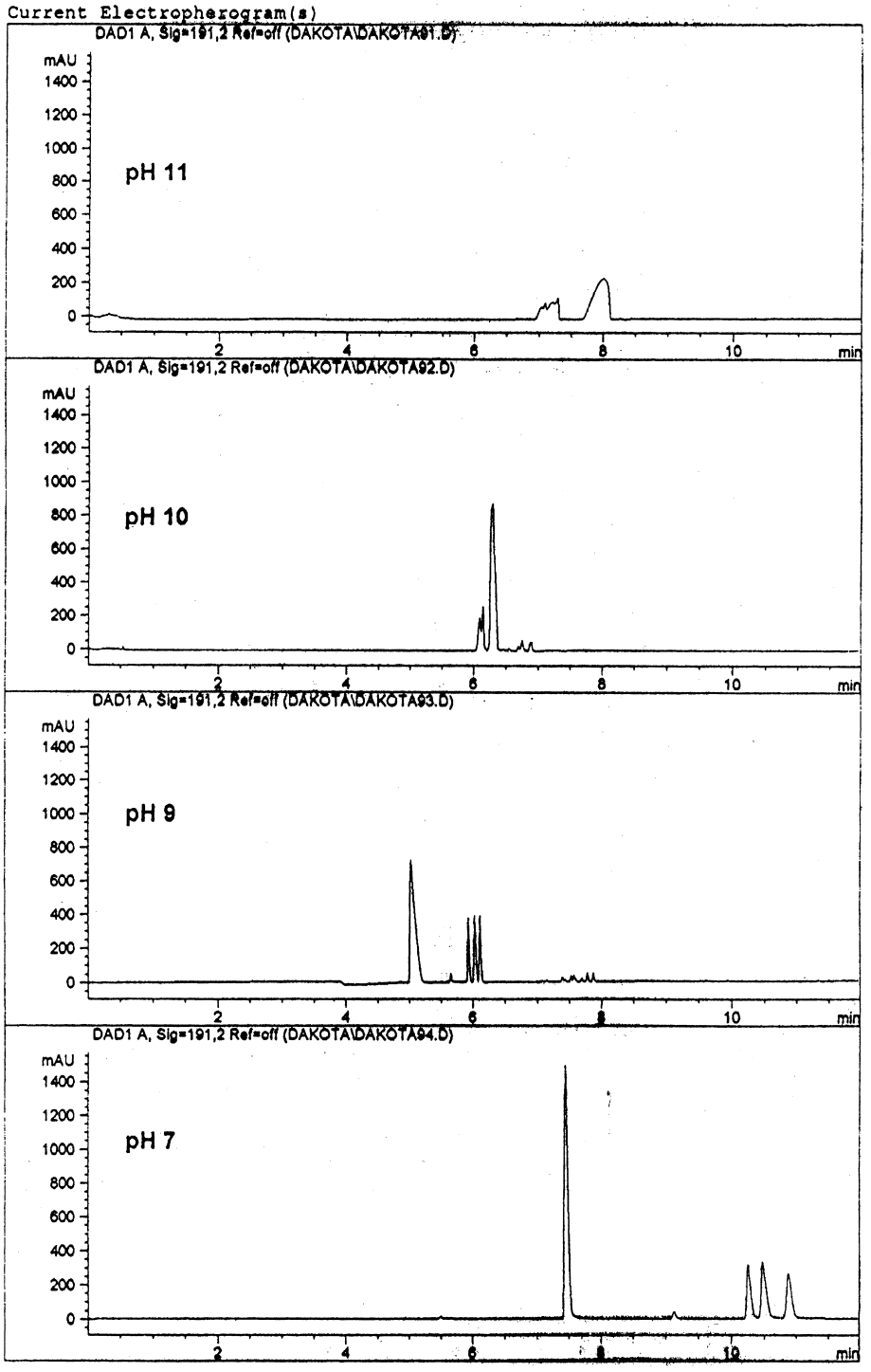

Fig. 1. MECC of phenol and cresol isomers at several buffer pH values. See text for experimental conditions.

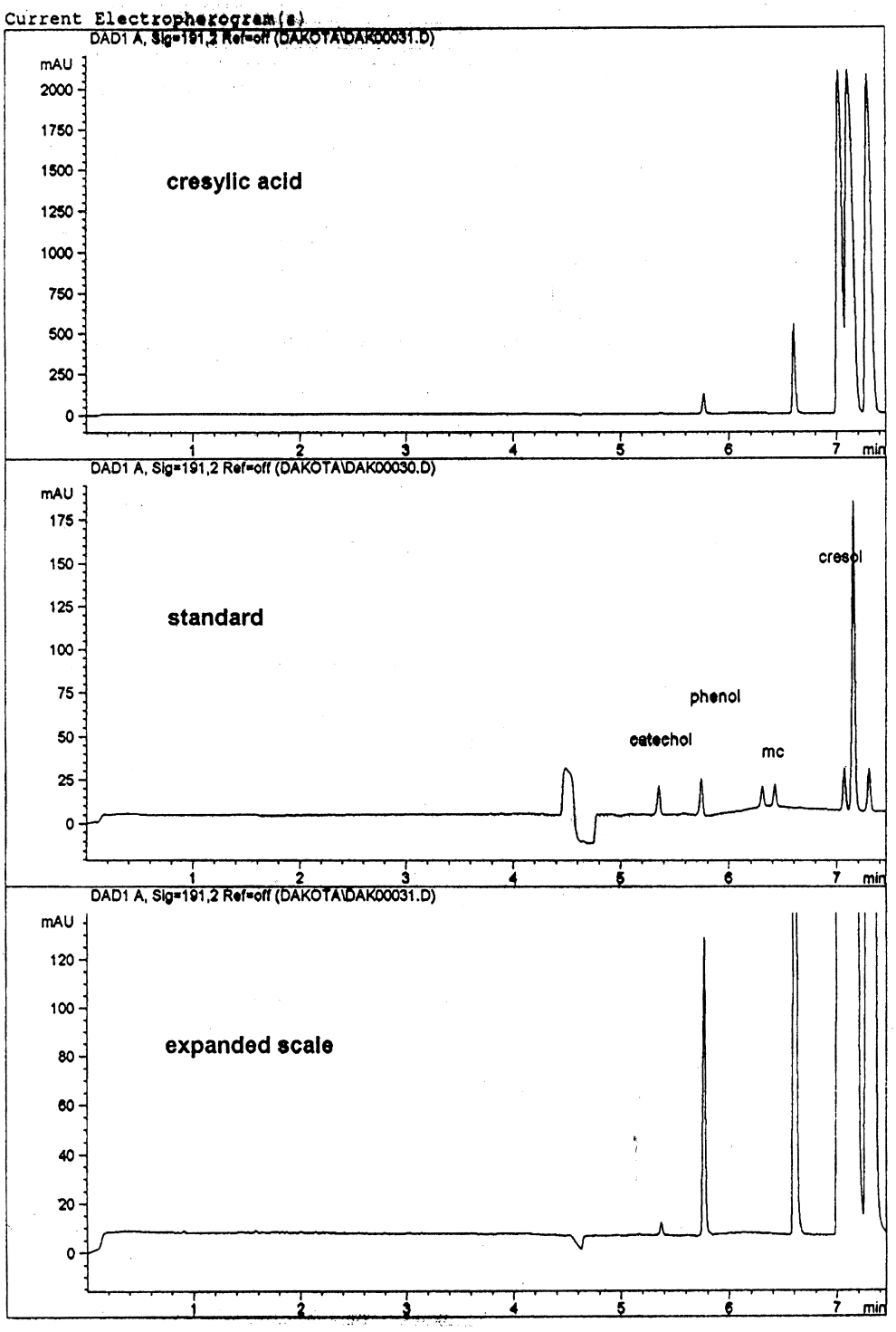

Fig. 2. MECC of cresylic acid at full and expanded scales.

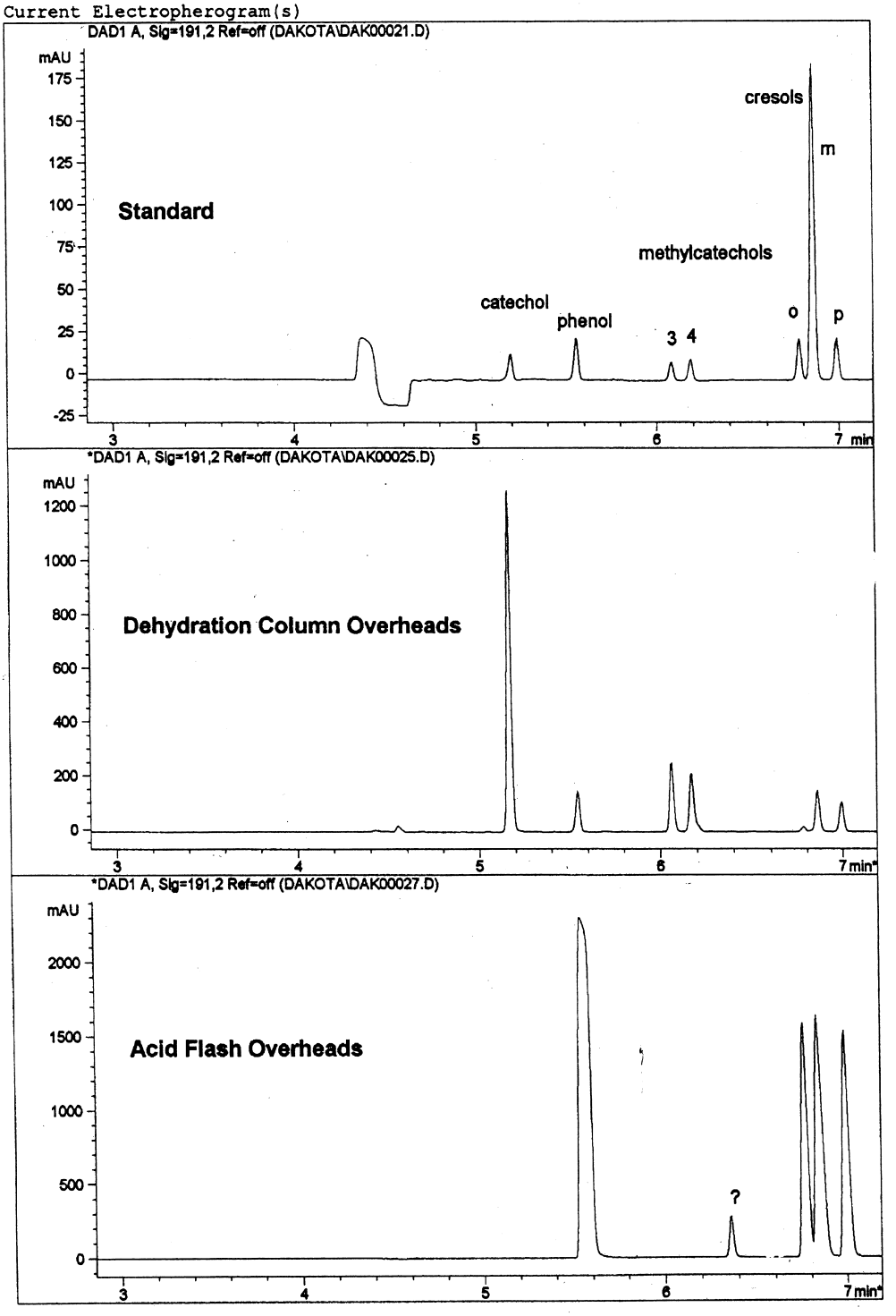

Fig. 3. MECC of dehydration column and acid flash overheads.

Table 1
Limits of detection and quantitation for phenolic impurities

Solute	LOD (ppm)	LOQ (ppm)
o-Cresol	0.25	0.84
p-Cresol	0.20	0.68
Phenol	0.72	2.4
Pyrocatechol	0.47	1.6
3-Methylcatechol	0.45	1.5
4-Methylcatechol	0.69	2.3

LOD measured at 3× baseline noise; LOQ measured at 10× baseline noise.

Table 2
Linear dynamic range

o-Cresol	8000
p-Cresol	6000
Phenol	8000
Pyrocatechol	12 000
3-Methylcatechol	13 000
4-Methylcatechol	8000

column washing and reequilibration between runs. By CE, a 3-min flush with BGE between runs is all that is required. A single CE method works for all matrices. As a result, one CE system has replaced four HPLC systems.

Table 3
Repeatability of analysis

Solute	Migration time (RSD, %)	Peak area ratio (RSD, %)
o-Cresol	1.76	1.43
p-Cresol	1.98	0.74
Phenol	0.73, 0.82	2.32, 0.56
Pyrocatechol	0.60, 0.66	0.28, 0.96
3-Methylcatechol	0.89, 1.09	2.58, 0.88
4-Methylcatechol	0.91, 1.14	1.37, 1.18

Data from five runs on 2 separate days.

3.4. Sample preparation

The HPLC sample preparation is a two-step method as described in Section 2.3. This procedure is required to solubilize neutral oils produced during synthesis. By CE, a simple one-step dilution with BGE solubilizes all material.

4. Conclusions

These studies have shown that it is possible to improve the LOD for impurities in bulk chemicals by their MECC separation as neutral compounds. The

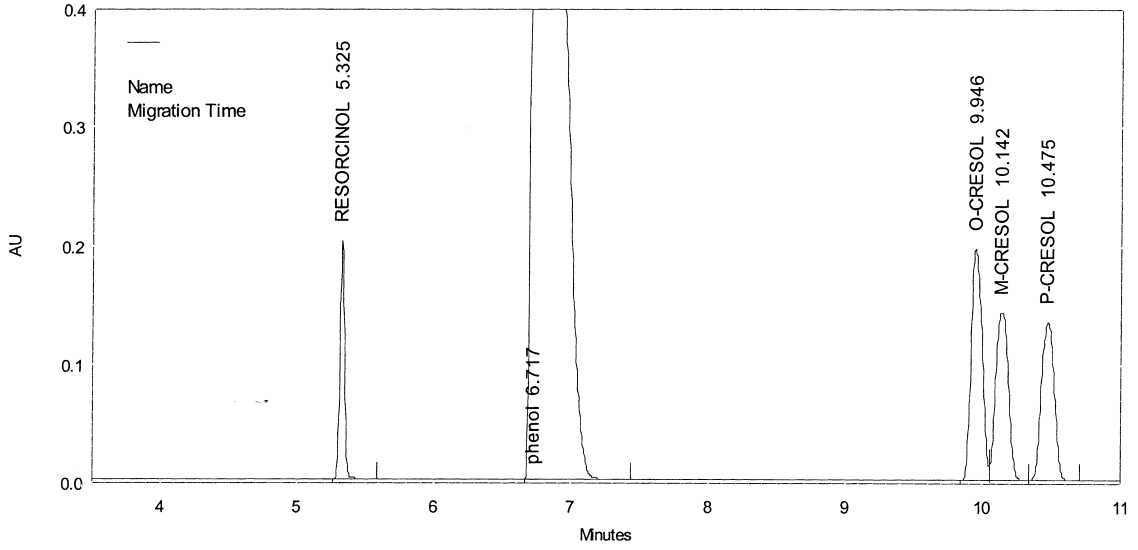

Fig. 4. MECC of high purity phenol spiked with 5 ppm of cresol isomers.

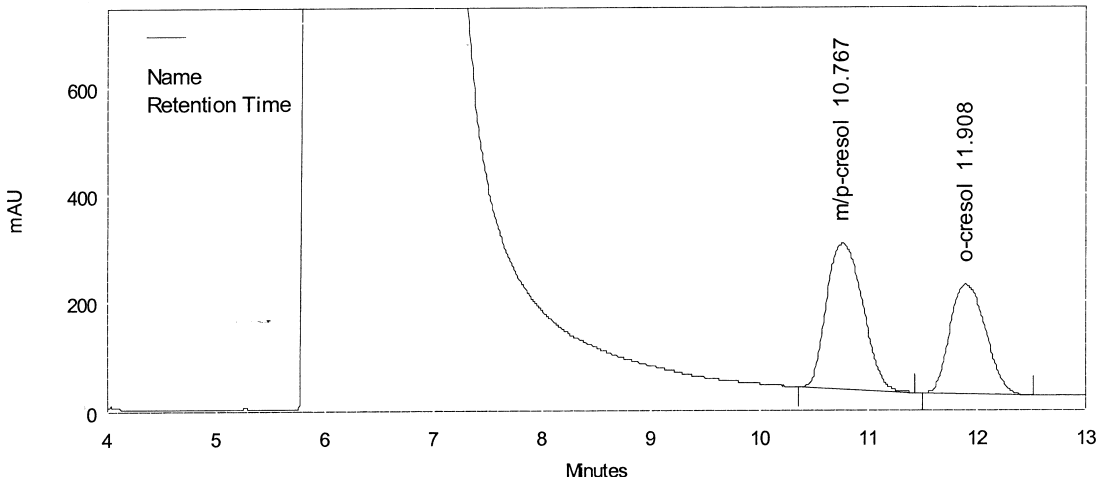

Fig. 5. HPLC of high purity phenol spiked with 200 ppm cresol isomers.

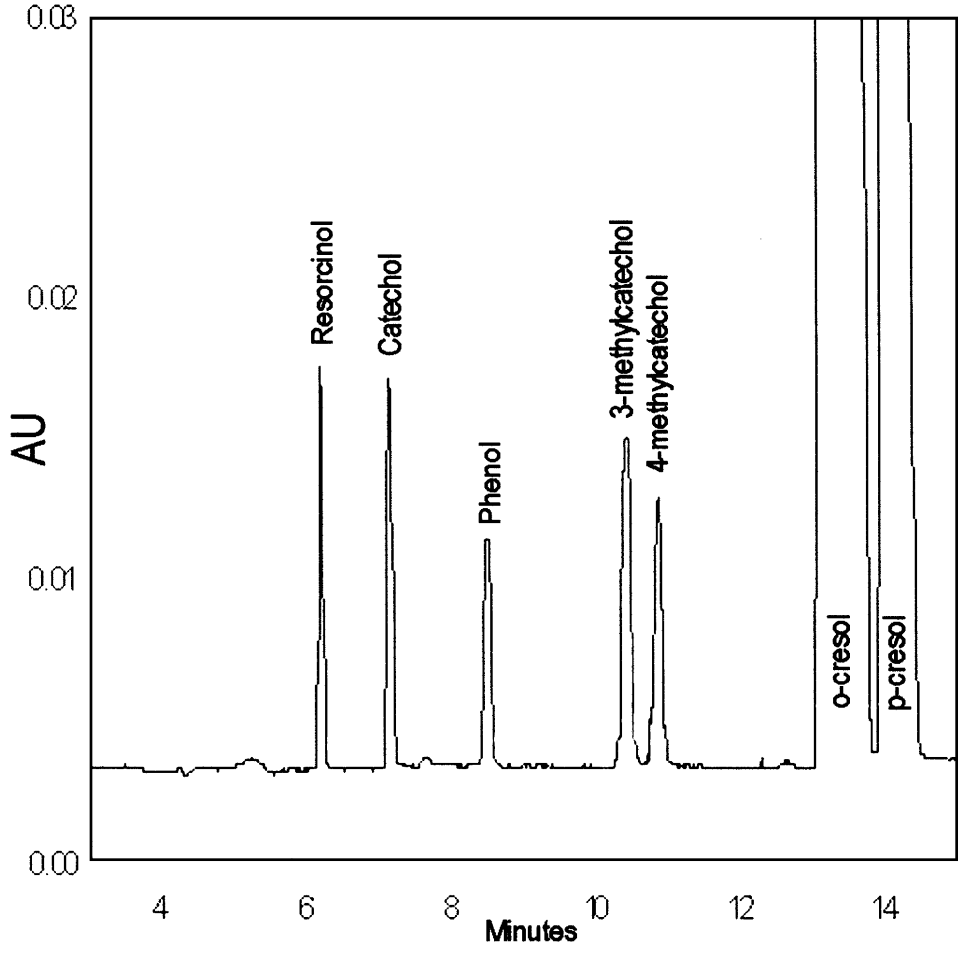

Fig. 6. MECC of cresylic acid spiked with 20 ppm of each impurity.

method operates around the clock, 7 days/week and is run by laboratory technicians. The LODs are sub-ppm, the linear dynamic range is about 10^5, sample preparation is simplified and four LC systems were replaced by one CE system. With some modifications, this method will be applicable to determine impurities in bulk pharmaceuticals.

References

[1] P. Gebauer, W. Thormann, P. Bocek, Electrophoresis 16 (1995) 2039.
[2] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[3] S. Terabe et al., Anal. Chem. 56 (1) (1984) 111.