Determination of *p*-Toluenesulfonic Acid in Water-Insoluble Drugs

Pan Guangwen,[1] Liao Dongli,[1] Xu Qun,[1] and Jeffrey Rohrer[2]
[1]Thermo Fisher Scientific, Shanghai, People's Republic of China;
[2]Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words
Organic Acid Analysis, Valve-Switching Technique, On-Line Cleanup, Drug Analysis, Pharmaceutical, Ion Chromatography

Goal
To develop an efficient ion chromatography (IC) method for the rapid and sensitive determination of *p*-toluenesulfonic acid in water-insoluble drugs

Introduction
p-Toluenesulfonic acid—an aromatic organic acid also known as tosylic acid—is widely used in the pharmaceutical industry as a counterion for basic drugs because of its strong acidic and hydrophilic properties. Therefore, for drug quality control and authenticity testing it is important to determine the content of *p*-toluenesulfonic acid in drug substances and products. The reported analytical methods used for *p*-toluenesulfonic acid in drugs are high-performance liquid chromatography (HPLC), liquid chromatography/mass spectrometry (LC/MS), nuclear magnetic resonance (NMR), and gas chromatography (GC).[1-4] Here, the convenience and accuracy of a Reagent-Free™ IC (RFIC™) approach is applied to the determination of *p*-toluenesulfonic acid in water-insoluble drugs using an on-line matrix-elimination as described in Application Note 220, albeit with different columns and a different system configuration.[5]

Equipment and Consumables
- Thermo Scientific™ Dionex™ ICS-2100 IC system with Degas (includes pump, eluent generator, injection valve, column heater, conductivity detector, and touchscreen)
- Thermo Scientific Dionex AS-AP Autosampler with optional valves (one 6-port valve and one 10-port valve for sequential injection)
- Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor, 4 mm (P/N 082540)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.1 or above
- Thermo Scientific™ Target2™ Polypropylene Syringe Filters, 0.45 µm, 30 mm (Fisher Scientific P/N F2502-9)

Reagents and Standards
- Deionized (DI) water, 18.2 M -cm resistivity (generated by a Thermo Scientific™ Barnstead™ GenPure™ Pro water purification system, P/N 50131948)
- Methanol, 99.8%, HPLC Grade (Fisher Scientific P/N AC610090040)
- Thermo Scientific Dionex AS22 Sodium Carbonate (Na_2CO_3)/Bicarbonate ($NaHCO_3$) Eluent Concentrate (100×), 250 mL (P/N 063965)*
- *p*-Toluenesulfonic Acid Monohydrate, Crystalline/Certified (Fisher Scientific P/N A320-500)

**Note: The ready-to-dilute Dionex AS22 Eluent Concentrate for the Thermo Scientific™ Dionex™ IonPac™ AS22 Analytical and Guard columns is available for anion applications as a 100× concentrate containing 0.45 M Na_2CO_3/0.14 M $NaHCO_3$.*

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Conditions	
On-Line Cleanup (Matrix Elimination)	
Column:	Dionex IonPac NG1 Guard, 10 µm, 4 × 35 mm (P/N 039567)
Eluent:	Methanol/water (80:20, v/v)
Flow Rate:	0.55 mL/min
Injection Volume:	25 µL
Temperature:	30 °C
Separation	
Columns:	Dionex IonPac AG22 Guard, 4 × 50 mm (P/N 064139) Dionex IonPac AS22 Analytical, 4 × 250 mm (P/N 064141)
Eluent:	4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$ (100-fold dilution of Dionex AS22 Eluent Concentrate)
Valve Switching:	Table 1
Flow Rate:	1.2 mL/min
Temperature:	30 °C
Detection:	Suppressed conductivity, recycle mode, 31 mA

Sample Preparation

Two powder samples were kindly donated by a biopharmaceutical customer in Suzhou, China, who explained that it is difficult to get satisfactory results when using water to directly extract *p*-toluenesulfonic acid from the drug powder. Therefore, methanol/water (80/20, v/v) was used as the extractant.

Dissolve 25 mg of a sample with 250 mL methanol/water (80/20, v/v) to obtain a 100 mg/L sample solution. Filter through a 0.45 µm syringe filter prior to injection.

Results and Discussion

Figure 1 shows the flow schematic of the on-line cleanup system, which is coupled to the analytical column using two valves, one 6-port and one 10-port. The filtered sample is directly injected by the autosampler onto the system and stored in Loop 1 (10_1 position on the 10-port valve); the analytical column is simultaneously equilibrated using Pump 2 (2_1 position on the 6-port valve). After the analytes are loaded in Loop 1, they are eluted using Pump 1 from Loop 1 (2_1 position on the 10-port valve) into Loop 2 (6_1 position on the 6-port valve) through the cleanup column, where interfering sample components are bound and analytes are not retained. After the analytes are captured in Loop 2, the loop is switched into the analytical flow path (2_1 position on the 6-port valve) to flush the captured analytes using Pump 2. The analytes are separated on the analytical column and then detected, while the 10-port valve remains in the 2_1 position to elute the initially bound sample components from the cleanup column using Pump 1.

The Dionex IonPac NG1 Guard column packed with a polymeric sorbent was evaluated as cleanup column for eliminating the water-insoluble components of the drug samples. Normally, water is ideal as an eluent that allows hydrophobic compounds to bind with polar compounds flushed out of the Dionex IonPac NG1 column; however, for samples with a water-insoluble matrix, a water eluent can decrease method reproducibility and accuracy. Therefore, methanol/water (80/20, v/v) was used here on the Dionex IonPac NG1 column to eliminate water-insoluble sample components, while allowing *p*-toluenesulfonic acid to flow through and into Loop 2 (500 µL). *p*-Toluenesulfonic acid was then delivered to the analytical flow (Figure 1).

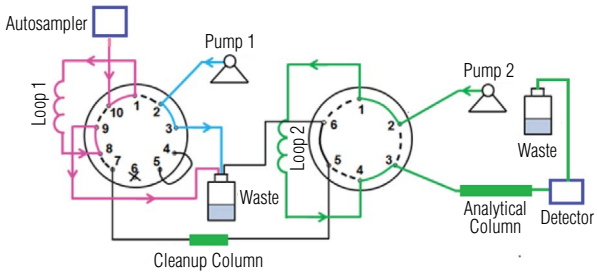

Figure 1. Flow schematic of the on-line cleanup system.

Table 1. Valve-switching time program.

Time (min)	10-Port Valve Position	6-Port Valve Position	Description
–3.0	10_1	2_1	Inject (deliver sample to Loop 1)
–1.0	2_1		Eliminate matrix through Dionex IonPac NG1 column
–0.9		6_1	Deliver analytes to Loop 2
0		2_1	Determine analyte and regenerate Dionex IonPac NG1 column
15	10_1		Return to initial status

Setting the valve-switching time to capture the *p*-toluenesulfonic acid in Loop 2 is the key to success of this on-line cleanup step. The proper time can be estimated by comparing a *p*-toluenesulfonic acid standard's peak area, obtained using this on-line cleanup technique, to that obtained using a traditional IC method that does not use the on-line cleanup step. Experiments show that when the running time of the elimination process (2_1 position on the 10-port valve and 6_1 position on the 6-port valve) is 0.1 min (Table 1, valve-switching time set at –1.0 and –0.9 min), the ratio of *p*-toluenesulfonic acid peak areas is 1.0—equivalent to the theoretical value—and thus demonstrates no loss of *p*-toluenesulfonic acid. As shown in Figure 2, *p*-toluenesulfonic acid in the drug samples can be separated without interference.

Good linearity was observed from 10 to 150 mg/L (10, 20, 50, 80, 100, and 150 mg/L) when plotting the concentration versus peak area. The linear regression equation was $A = 0.0255c - 0.0754$, where A represents peak area, c represents concentration of the analyte, and the coefficient of determination was 0.9993. This calibration curve was used to quantify *p*-toluenesulfonic acid in drug samples. Although it was not important for this assay, the authors determined that the method detection limit was 1 mg/L using a signal-to-noise ratio = 3. Using the IC on-line cleanup method, the determined amounts of *p*-toluenesulfonic acid were 26.9% in Drug Sample 1 and 26.6% in Drug Sample 2, which were accurate as confirmed by the customer.

Conclusion

This study describes an efficient IC method with on-line sample cleanup to determine *p*-toluenesulfonic acid in water-insoluble drugs. On-line cleanup provides the benefits of efficient matrix elimination, time savings, and convenience.

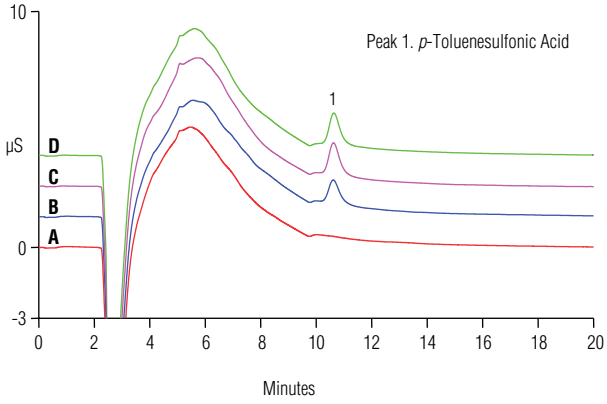

Figure 2. Separation of *p*-toluenesulfonic acid in (A) a blank, (B) *p*-toluenesulfonic acid standard (20 mg/L), (C) Drug Sample 1, and (D) Drug Sample 2.

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