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# Determination of Ethyl Sulfate Impurity in Indinavir Sulfate Drug Using Ion Chromatography

## **INTRODUCTION**

Indinavir sulfate is a specific and potent inhibitor of HIV-1 protease and is widely used in the treatment of AIDS.<sup>1</sup> Indinavir sulfate is prepared by dissolving indinavir base in anhydrous ethanol that is then treated with sulfuric acid. At ambient temperature, a mixture of ethanol and sulfuric acid reacts to yield monoethylsulfate (ethyl sulfate). Therefore, treatment of indinavir base in anhydrous ethanol with sulfuric acid is performed at a controlled temperature of less than 0 °C to avoid the formation of ethyl sulfate.<sup>1</sup> However, it is very important for manufacturers of drug products to monitor the level of anticipated process-related and degradation impurities before commercial release to prove the consistency of the manufacturing process. It is also important to monitor drug stability during storage.

Ion chromatography (IC) has been successfully used to measure ionic drug degradation products and process-related impurities. *N*-methylpyrrolidine, a degradation product of cefipime, has been measured in cefipime and simulated Cefipime for Injection.<sup>2,3</sup> In addition, IC also has been used to determine the process impurity ethylhexanoate in clavulanate.<sup>4</sup>

The work here shows an IC method to measure ethyl sulfate in indinavir sulfate. This method uses a Thermo Scientific Dionex IonPac® AS12A column with a carbonate/bicarbonate eluent to separate ethyl sulfate from other anions, including sulfate. The eluent was produced using an eluent generator to improve ease of use and retention time reproducibility.

Reproducible chromatography is demonstrated without the labor or possible error of eluent preparation, and without the need to prepare sulfuric acid solutions for suppressed conductivity detection.

## **EQUIPMENT**

Thermo Scientific Dionex ICS-3000 or Dionex ICS-5000 system including:\*

DP Dual Pump

DC Detector/Chromatography module with dual-temperature zone equipped with 6-port injection valves

EG Eluent Generator module

EPM Electrolytic pH Modifier

Thermo Scientific Dionex Chromeleon® Chromatography Data System software, Version 6.80, SR9

\*A Dionex ICS-2100 or other Dionex IC system featuring eluent generation also can be used. Alternately, if the eluents are manually prepared, any Dionex IC system can be used.

## **REAGENTS AND STANDARDS**

Deionized (DI) water, Type I reagent grade, 18 MΩ-cm resistivity or better

Dionex Seven Anion Standard II (P/N 57590)

Sulfuric acid, 98% (H<sub>2</sub>SO<sub>4</sub>, Merck)

Ethanol (Absolute), AR grade (C<sub>2</sub>H<sub>5</sub>OH, RCI Labscan)

## **PREPARATION OF SOLUTIONS AND REAGENTS**

### **Carbonate (2.7 mM)/Bicarbonate (0.3 mM) Eluent**

Generate carbonate/bicarbonate eluent on-line by pumping high-quality DI water (18 M $\Omega$ -cm resistivity or better) through the Thermo Scientific Dionex EluGen EGC II K<sub>2</sub>CO<sub>3</sub> Cartridge and EPM. Use Chromeleon software to control the concentration of carbonate/bicarbonate. Add backpressure tubing to achieve 2000–2300 psi system backpressure, which allows the EG degasser to work properly. Refer to the Dionex ICS-3000 operator's manual for how to add backpressure. Alternately, prepare the eluent manually by pipetting 5.4 mL of 0.5 M Na<sub>2</sub>CO<sub>3</sub> (P/N 037162) and 0.6 mL of 0.5 M NaHCO<sub>3</sub> (P/N 037163) into a 1 L volumetric flask and dilute to volume with DI water.

Eluent generation was used to produce the data in this study.

### **Ethyl Sulfate Stock Solution**

Transfer 0.5 mL ethanol into a 100 mL volumetric flask. Slowly add 0.5 mL 98% sulfuric acid to the same volumetric flask. Gently swirl the flask to mix and bring to volume with DI water. Note: this preparation must be performed in a fume hood while wearing goggles and protective clothing.

### **Mixture of Seven Anion Secondary Standard**

Add 0.5 mL Dionex Seven Anion Standard Stock Solution to a 10 mL volumetric flask and bring to volume with DI water.

### **Ethyl Sulfate Standard Solution**

Add 0.1 mL ethyl sulfate stock solution to a 25 mL volumetric flask and bring to volume with DI water.

### **Sample Preparation**

Dissolve a capsule of the indinavir sulfate drug in a 10 mL volumetric flask with DI water. Filter the sample solution with a 0.45  $\mu$ m syringe filter.

## **CONDITIONS**

Column:	Dionex IonPac AS12A (4 × 200 mm) (P/N 046034)
Guard:	Dionex IonPac AG12A (4 × 50 mm) (P/N 046035)
Eluent Source:	Dionex EluGen EGC II K <sub>2</sub> CO <sub>3</sub> cartridge (P/N 058904) with EPM Electrolytic pH Modifier and EGC Carbonate Mixer, 4 mm (P/N 061686)
Eluent:	2.7 mM Carbonate/0.3 mM bicarbonate
Flow Rate:	1.5 mL/min
Inj. Volume:	20 $\mu$ L
Column Temp.:	30 °C
Pressure:	~2300 psi
Detection:	Suppressed conductivity, Thermo Scientific Dionex ASRS <sup>®</sup> 300, 4 mm (P/N 064554), recycle mode, current 25 mA
Background:	~11 $\mu$ S

## **RESULTS AND DISCUSSION**

### **Ethyl Sulfate Production**

Ethyl sulfate is not commercially available, but it can be easily produced in the laboratory by mixing sulfuric acid and ethanol.<sup>5</sup> Ethyl sulfate was prepared as described in the Ethyl Sulfate Stock Solution section, and ultimately used to prepare the Ethyl Sulfate Standard Solution used for the chromatography in this study. IC was used to judge the success of ethyl sulfate production. The Ethyl Sulfate Standard Solution was injected into the IC system and the chromatography was compared to ethanol and sulfuric acid (diluted in the same manner as was the Ethyl Sulfate Stock Solution to prepare the Ethyl Sulfate Standard Solution [Figure 1]). The Ethyl Sulfate Standard Solution exhibited two peaks: one at approximately 5 min and another at the retention time of sulfate. No peaks were found in the ethanol solution and only a peak corresponding to sulfate was found in the sulfuric acid solution. Therefore, the peak at approximately 5 min was assumed to be ethyl sulfate.

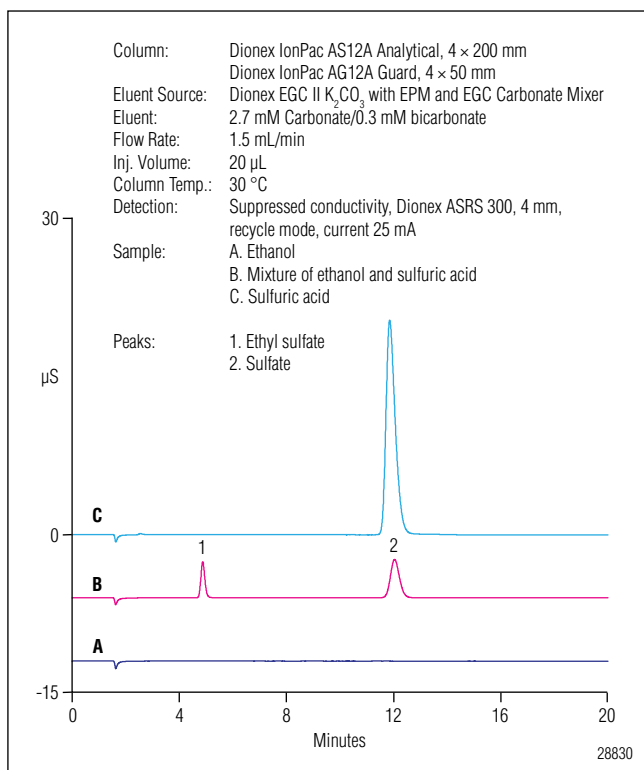


Figure 1. Overlay of chromatograms of ethanol, sulfuric acid, and a mixture of ethanol and sulfuric acid.

## Separation

The Dionex IonPac AS22, AS23, and AS12A columns were evaluated to determine the best separation of ethyl sulfate from the other inorganic anions. The Dionex IonPac AS12A column provided the best separation. Figure 2 shows chromatograms of a mixture of seven common inorganic anions and the prepared Ethyl Sulfate Standard Solution. Note that ethyl sulfate was well resolved from the other anions. The Ethyl Sulfate Standard Solution was injected five times to evaluate stability. The peak area was stable and there was no noticeable increase or decrease in retention time (Table 1).

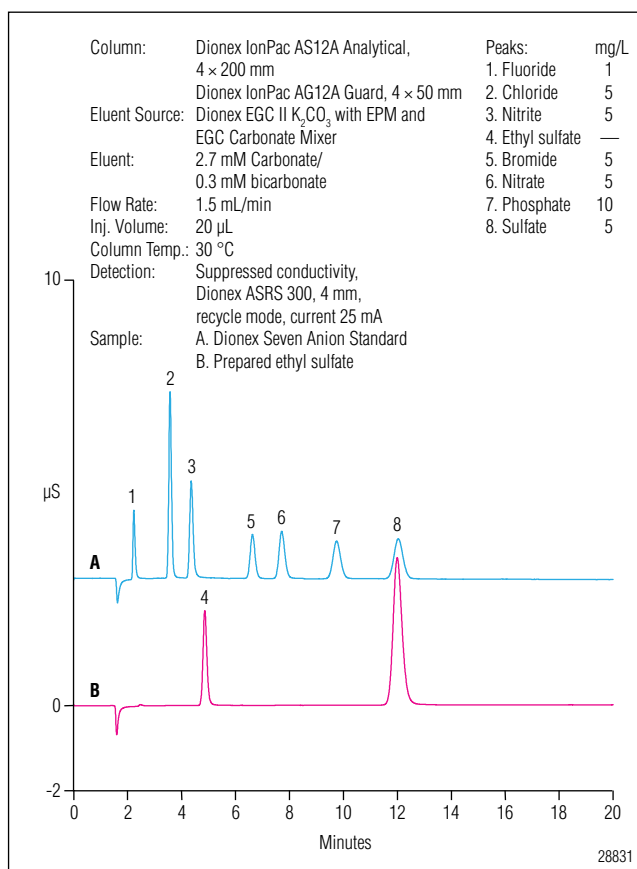


Figure 2. Chromatograms of a Dionex Seven Anion Standard and the prepared ethyl sulfate.

Table 1. Area of Ethyl Sulfate Standard Solution	
Injection No.	Area (µS*min)
1	0.3793
2	0.3736
3	0.3736
4	0.3836
5	0.3761
<b>Average</b>	<b>0.3772</b>
<b>% RSD</b>	<b>1.14</b>

## Sample Analysis

A customer kindly provided the indinavir sulfate final drug product in the form of capsules that had been stored on a shelf for a long period of time prior to the analysis reported here. Five capsules of sample were prepared and each sample was injected five times into the IC system. Figure 3 shows the chromatography of one of the capsule samples. The putative ethyl sulfate peak was well resolved from the other peaks in the sample. Spiking the sample solution with the Ethyl Sulfate Standard Solution confirmed that the peak in the sample had the same retention time as the peak in the standard (Figure 4). The position of the peak tentatively identified as ethyl sulfate relative to sulfate and other peaks in the capsule sample was also consistent with a peak identified as ethyl sulfate in a publication that uses a longer IC method.<sup>1</sup> Peak area data from the 25 sample injections are shown in Table 2. These data show that ethyl sulfate was found in all five capsules and the peak area response was reproducible. There was also a consistent amount of ethyl sulfate in the five capsules.

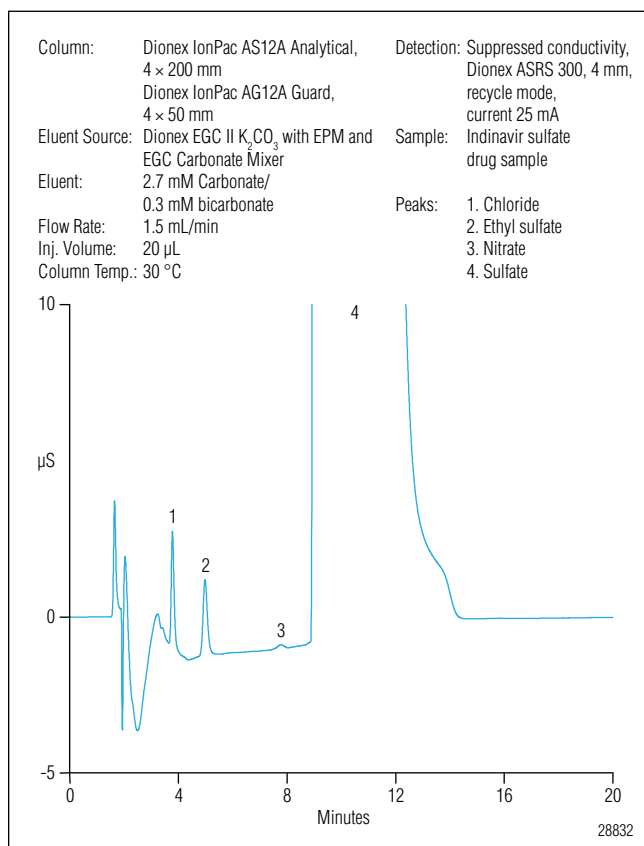


Figure 3. Chromatogram of the indinavir sulfate drug sample.

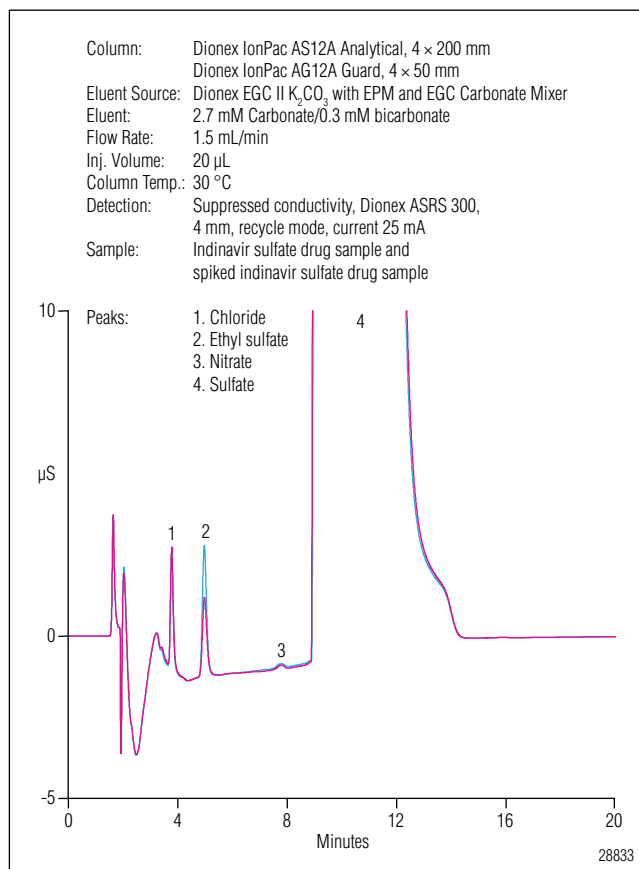


Figure 4. Overlay chromatograms of the indinavir sulfate and spiked indinavir sulfate sample.

Injection No.	Area (µS*min)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	0.5063	0.5189	0.4852	0.5037	0.4988
2	0.5015	0.5104	0.4812	0.4941	0.4994
3	0.4993	0.5115	0.4816	0.4930	0.4953
4	0.5098	0.5083	0.4827	0.4870	0.4919
5	0.5054	0.5087	0.4807	0.4887	0.4895
<b>Average</b>	<b>0.5045</b>	<b>0.5116</b>	<b>0.4823</b>	<b>0.4933</b>	<b>0.4950</b>
<b>RSD</b>	<b>0.82%</b>	<b>0.84%</b>	<b>0.38%</b>	<b>1.32%</b>	<b>0.87%</b>

## CONCLUSION

This work shows IC separation of ethyl sulfate in an indinavir sulfate drug sample. This method only requires the addition of DI water to the IC system to deliver reproducible chromatography while avoiding the time, labor, and potential error of manual eluent preparation. This method uses an electrolytic suppressor in recycle mode, and therefore does not require the analyst to prepare sulfuric acid solutions for eluent suppression prior to conductivity detection.

## REFERENCES

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