DIONEX

Application Note 262

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Determination of 2-Ethylhexanoic Acid Impurity in Clavulanate

INTRODUCTION

Clavulanic acid is a potent beta-lactamase inhibitor that when used in combination with penicillin and cephalosporin antibiotics, increases their effectiveness by counteracting bacterial resistance.[1, 2] Clavulanic acid is produced from *Streptomyces clavuligerus* and then isolated by acidifying and extracting the cold, clarified culture medium with ethyl acetate.[3] Further preparation of clavulanic acid is needed to increase purity, minimize aqueous hydrolysis, and convert the acid to a stable form used in the pharmaceutical product.[4] Potassium clavulanate is more stable than the free acid or other salts such as the sodium, calcium, or magnesium analogs. One possible secondary purification approach uses a non-aqueous precipitation of potassium clavulanate by adding the potassium salt of 2-ethylhexanoic acid in isopropanol to the primary isolation extract.[5] Although 2-ethylhexanoic acid is soluble in the organic solvents, a small fraction may co-precipitate with the potassium clavulanate active pharmaceutical ingredient (API).

The United States Pharmacopeia (USP) monograph for potassium clavulanate describes a procedure to determine 2-ethylhexanoic acid in a 75 mg/mL solution of the API in a strong acid that is extracted into organic solvent and analyzed by gas chromatography with flame ionization detection.[6] The work shown here describes a simpler approach for determining 2-ethylhexanoic acid in clavulanate using a Reagent-Free™ Ion Chromatography (RFIC™) system. Clavulanate USP reference standard, prepared at 0.5 mg/mL in deionized water, was spiked with 2-ethylhexanoic acid and injected directly on an IonPac® AS11 column without additional sample pretreatment. The target analyte was separated from clavulanate-related peaks using electrolytically generated potassium hydroxide eluent and measured using suppressed conductivity detection.

EQUIPMENT

Dionex ICS-2100 system* including:

- Single isocratic pump
- Vacuum degasser
- Eluent generator
- High pressure, 6-port injector
- Column heater enclosure
- Conductivity cell and detector
- EO Eluent Organizer, including pressure regulator, and 2 L plastic bottle
- AS Autosampler and 2 mL vial tray
- Chromeleon® Chromatography Data System (CDS) Software Version 6.8 or 7
- Helium or nitrogen; 4.5-grade (99.995%) or better, < 5 ppm oxygen (Praxair)

Filter unit, 0.2 µm nylon (Nalgene® 90 mm Media-Plus, Nalge Nunc International P/N 164-0020) or equivalent nylon filter

Vacuum pump (Gast Manufacturing Corp. P/N DOA-P104-AA) or equivalent, for degassing eluents

1.5 mL Polypropylene injection vials with caps (Vial Kit, Dionex P/N 061696)

*This application can be run using any Dionex RFIC system

CONSUMABLES

EluGen II KOH Cartridge (Dionex P/N 058900)

CR-ATC (Dionex P/N 060477)

ASRS® 300 suppressor, 2 mm (Dionex P/N 064555)

IonPac AG11 column, 2 × 50 mm (Dionex P/N 044079)

IonPac AS11 column, 2 × 250 mm (Dionex P/N 044077)

REAGENTS AND STANDARDS

Deionized water, 18 MΩ-cm resistance or higher, filtered and degassed

Clavulanate Lithium Reference Standard (USP P/N 1134426)

2-Ethylhexanoic acid, 99% (VWR P/N 101226-112)

CONDITIONS

Columns: IonPac AG11, 2 × 50 mm (Dionex P/N 044079)
IonPac AS11, 2 × 250 mm (Dionex P/N 044077)

Eluent: 3 mM KOH from -10 to 0 min (column equilibration), 3 mM KOH from 0 to 10 min (separation), 3 to 60 mM KOH from 10 to 10.1 min, 60 mM KOH from 10.1 to 20.1 min (column cleanup)

Flow Rate: 0.25 mL/min

Inj. Volume: 5 µL

Column Temp.: 30 °C

Detection: Suppressed conductivity, ASRS 300 suppressor, 2 mm (Dionex P/N 064555), recycle mode, 2 mA suppressor current during equilibration and separation, switch to 38 mA during column cleanup

Background: <1 µS at sample injection

Noise: <2 nS/min

Backpressure: 2500 psi

Run Time: 20.1 min

Time (min)	[KOH] (mM)	Suppressor Current (mA)	Comment
-10	3	2	Equilibrate column
0	3	2	Inject sample
10	3	2	Finish separation
10.1	60	38	Start column cleanup
20.1	60	38	Complete column cleanup

STANDARDS AND SURROGATE SAMPLE SOLUTIONS

500 µg/mL Potassium Clavulanate Matrix Solution

Dissolve 0.0432 g of lithium clavulanate reference standard in 100.0 mL of degassed deionized water to make 432 µg/mL of solution, which is equivalent to 500 µg/mL of the potassium salt form (the factor for converting equimolar amounts of lithium salt to potassium salt is 1.157). Store in a high-density polyethylene or polypropylene bottle at 4 ºC.

1000 µg/mL 2-Ethylhexanoic Acid Stock Solution

Dissolve 0.1000 g of 2-ethylhexanoic acid in ~ 75 mL of degassed deionized water by stirring vigorously overnight at 30 to 35 ºC. Let the solution cool to room temperature, remove the stirring bar, and wash the stirring bar with water into the solution, making sure that the total solution volume does not exceed 100 mL. Add degassed deionized water for a total solution volume of 100.0 mL. Store in a high-density polyethylene or polypropylene bottle at 4 ºC.

Make all subsequent dilutions of 2-ethylhexanoic acid stock standard gravimetrically with degassed deionized water when generating standards for calibration and limit of detection/limit of quantification (LOQ/LOQ) studies, or with 500 µg/mL potassium clavulanate matrix solution when generating surrogate samples for accuracy and precision studies.

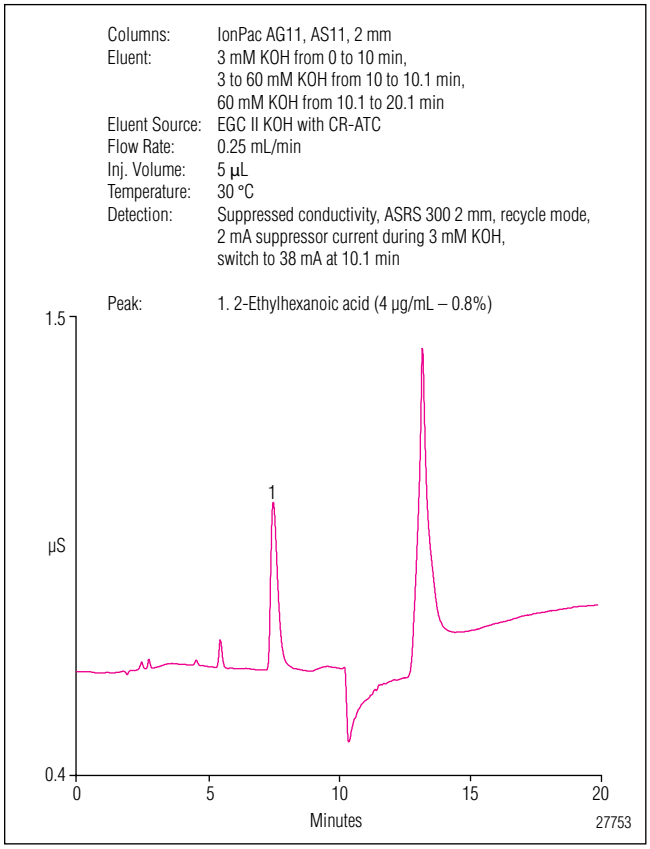

Figure 1. Chromatogram of 4 µg/mL 2-ethylhexanoic acid in DI water.

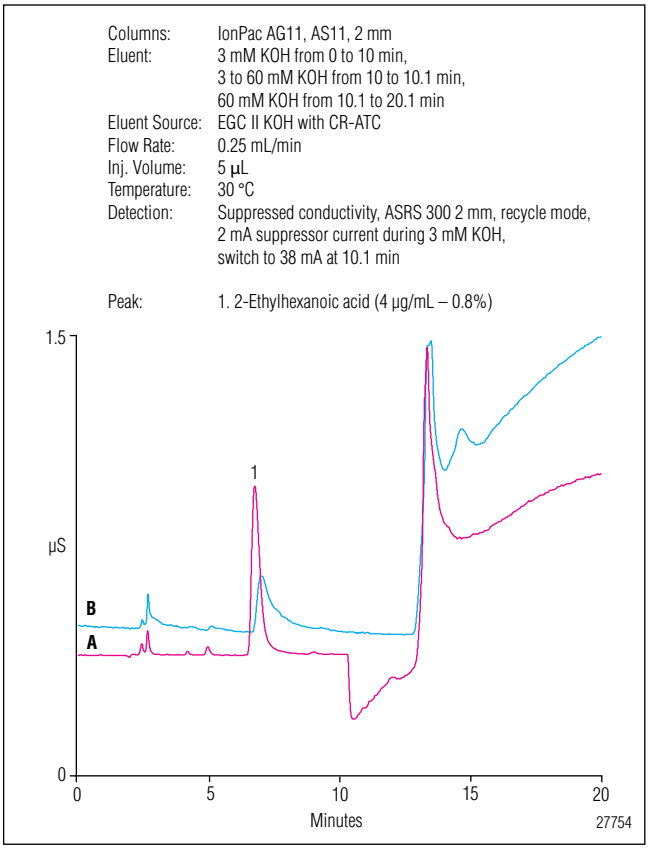

Figure 2. The effect of high versus low suppressor current during the separation of 2-ethylhexanoic acid. Trace A: 2 mA with step-up to 38 mA at 10 min; Trace B: 38 mA constant current.

RESULTS AND DISCUSSION

Chromatography

In AU 157, the IonPac AS17 column was used to separate potential anionic contaminants, such as 2-ethylhexanoic acid, in electronic component extracts.[7] Although the IonPac AS17 column previously was a good choice for 2-ethylhexanoic acid determinations in clean, well-characterized matrices, it was not chosen for this application because of its low capacity.[8] The IonPac AS11 column was selected for its ability to separate a wide range of inorganic and organic anions in complex matrices.[9] In addition, the IonPac AS11 column has 30% higher capacity than the IonPac AS17 column and lower surface hydrophobicity, a property that is expected to produce better peak shape for 2-ethylhexanoic acid.

Figure 1 shows a chromatogram of a 4 µg/mL 2-ethylhexanoic acid standard prepared in deionized water. This concentration corresponds to the 0.8% acceptance criterion cutoff value specified in the USP monograph when the potassium clavulanate concentration is 500 µg/mL.[6] The chromatographic features in Figure 1 observed after 10 min were also present in the system (no injection) and DI water blanks. The baseline dip was due to increasing the suppressor current at 10.1 min (Trace A in Figure 2). When a higher suppressor current was maintained throughout the chromatographic analysis, significant peak tailing for 2-ethylhexanoic acid was observed (Trace B in Figure 2). Therefore, the authors strongly recommend using the optimal current setting for the corresponding KOH concentrations.

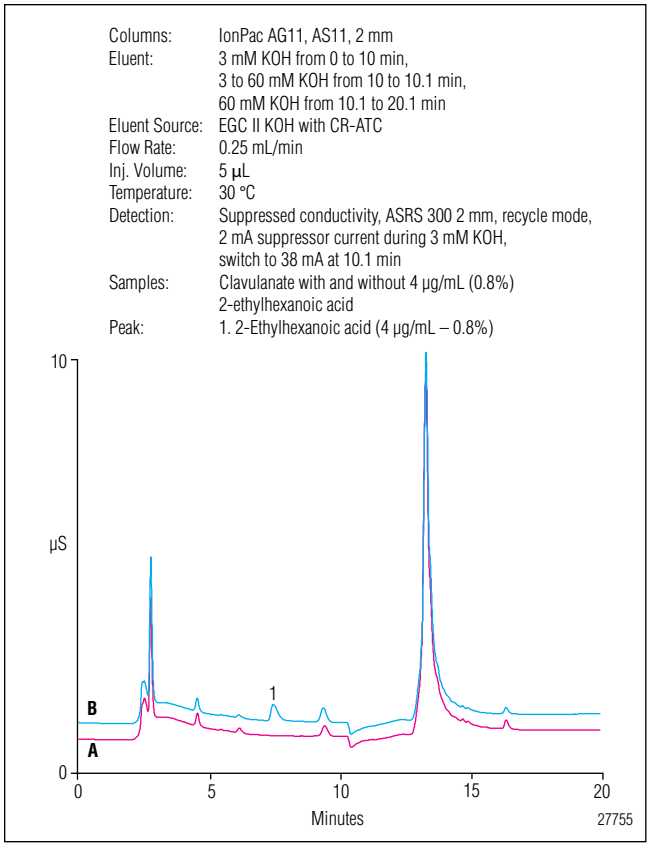

Figure 3. Chromatographic comparison of clavulanate A) without and B) with 4 µg/mL (0.8%) 2-ethylhexanoic acid.

Potassium clavulanate and its decomposition products do not interfere with the chromatography of 2-ethylhexanoic acid (Figure 3). The 10 min cleanup with 60 mM KOH was determined to be sufficient to elute the matrix components from the column and, therefore, prevent carryover in subsequent injections.

Limit of Detection, Limit of Quantification, and Linearity

The USP general chapter for validation <1225> suggests a signal-to-noise (S/N) ratio of three for LOD and 10 for LOQ.[10] Baseline noise was determined to be 1.4 nS by measuring the peak-to-peak noise of seven system (no injection) blanks over a 2 min window centered on the retention time of 2-ethylhexanoic acid. Peak heights from triplicate injections of standards in DI water were plotted versus 2-ethylhexanoic acid concentration. The LOD and LOQ estimates for 2-ethylhexanoic acid were 0.036 and 0.12 µg/mL, respectively, corresponding to 0.0072 and 0.024% in 500 µg/mL potassium clavulanate. The LOQ estimate is more than 30-fold lower than the 0.8% acceptance criterion cutoff level.

Table 1. Retention Time and Peak Area Precisions for 0.8% 2-Ethylhexanoic Acid in Clavulanate API[a]

Day	N	Average Retention Time (min)	Retention Time RSD	Average Peak Area (µS*min)	Peak Area RSD
1	15	7.047	0.11	0.1154	2.0
2	15	7.010	0.18	0.1140	1.2
3	15	6.968	0.13	0.1141	0.9
4	15	6.927	0.06	0.1141	1.1
5	15	6.898	0.16	0.1126	0.7

[a]2-Ethylhexanoic acid concentration at the acceptance criterion cutoff level.

To determine method linearity, triplicate injections were made of calibration standards prepared at seven concentration levels in the range of 1 to 7 µg/mL of 2-ethylhexanoic acid, corresponding to 0.2 to 1.4% in 500 µg/mL potassium clavulanate. A plot of peak area versus concentration produced a correlation coefficient (r^2) value of 0.9991 using a linear least squares regression fit. The relative standard deviation of the measured peak areas from the areas predicted by the calibration equation was < 1.5%.

Accuracy and Precision

Method accuracy was evaluated by spiking 2-ethylhexanoic acid at three different concentrations in 500 µg/mL potassium clavulanate matrix solutions. Spiked 2-ethylhexanoic acid concentrations were 2.0 µg/mL (0.40%), 4.0 µg/mL (0.80%), and 6.0 µg/mL (1.2%). The average recoveries for seven replicates were 94.1 ± 1.7%, 99.0 ± 2.2%, and 100.0 ± 1.0%, respectively. No measurable peak area was detected at the 2-ethylhexanoic acid retention time with potassium clavulanate matrix blank injections.

Method reproducibility was evaluated with replicate injections of 4.0 µg/mL (0.80%) 2-ethylhexanoic acid in potassium clavulanate (the acceptance criterion cutoff value). Retention times and peak area precisions for 2-ethylhexanoic acid were determined from 15 replicate injections per day for 5 days. Table 1 shows daily retention time and peak area values and their respective RSDs. Peak area RSDs ranged from 0.7 to 2.0%, whereas the retention times for 2-ethylhexanoic acid trended lower on a daily basis by 0.4%, implying loss of column capacity. Running a daily check sample and adjusting the 2-ethylhexanoic acid retention time

window in Chromeleon software will assure proper peak assignment and integration for this potential impurity. If the 2-ethylhexanoic acid retention time decrease exceeds internal quality control requirements, replace the guard column. If no requirements are specified, replace the guard column after no more than 150 injections. For long-term storage (> 1 week), flush the column set with 100 mM sodium borate solution as described in the IonPac AS11 column product manual.[9]

PRECAUTIONS

Contact of clavulanate powder or 2-ethylhexanoic acid with eyes, skin, and respiratory tract causes irritation. The 2-ethylhexanoic acid also may be teratogenic. Wear protective gloves, chemical safety goggles, and a laboratory coat. To dispose of these materials, contact a licensed waste disposal service. Read the material safety data sheet for these compounds before use.

Extensive use of the column may cause performance degradation, such as loss of capacity, loss of resolution, shortened retention times, higher noise and background, spurious peaks, and peak tailing. Prescribed column cleanings did not restore 2-ethylhexanoic acid retention time. For more information on column troubleshooting, see the IonPac AS11 column product manual.[9]

CONCLUSION

This work presents an IC method for the detection and quantification of 2-ethylhexanoic acid, a potential impurity in potassium clavulanate API incorporated during sample purification. Results for LOD/LOQ, linear calibration range, spike recovery, retention time precision, and peak area precision determinations show that IC is an accurate and reproducible technique to determine 2-ethylhexanoic acid in clavulanate below the 0.8% acceptance criteria. The method uses 150-fold lower concentration of the API and eliminates the need for solvent extraction per the current USP monograph, thus consuming less of the API and simplifying sample preparation.

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SUPPLIERS

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