Ethacrynic Acid and Related Compounds

From Particulate to Monolithic Column

Currently there is no impurity profiling method in USP for ethacrynic acid, **thus a new impurity profiling method would require method submission to authorities**. The assay method uses a 300x3.9 mm column that contains packing L1 (RP-18). Flow rate is about 1 mL per minute, and column efficiency determined from the analyte peak is not less than 1200 theoretical plates. The tailing factor for the analyte peak is not more than 2; the capacity (retention) factor, is not less than 0.8. Using these performance criteria, we developed an in-house method for ethacrynic acid and its seven impurities at one of our application laboratories. This new method was thereafter transferred within the scope of allowed monograph method changes, see page 8.
In the original impurity profiling method, see page 13, the liquid chromatograph should be equipped with 280 nm detector and a 250x4.6 mm column that contains packing L1 (RP-18). No specific particle size is mentioned, thus any type of column backbone can be used.

To comply with pharmacopoeial changes and perform only partial revalidation this method can be changed by:

- Reduction of particle size to maximum 2.5 µm (50% since method uses a 5 µm particle in written standard operating procedure - SOP) or use a monolithic column
- Shortening the column to 75 mm (70%)
- Reduction of inner diameter if linear velocity is kept constant
- Reduction of injection volume as long as limit of detection (LOD) and linearity is OK.

A monolithic Chromolith® HighResolution RP-18 endcapped 100x4.6 mm column was chosen as the alternative for ethacrynic acid and its related impurities, see page 14.

The alternative column met the performance criteria so why should you change?

1. **The method will run faster** (Time-saving: 30 minutes per sample)
   (yes...the column length is 60% shorter which is also the percentage of shortening of column).

2. **Better overall peak symmetry for Ethacrynic acid**
   (indicate a better loadability for ethacrynic acid on the monolithic over the particle packed column)

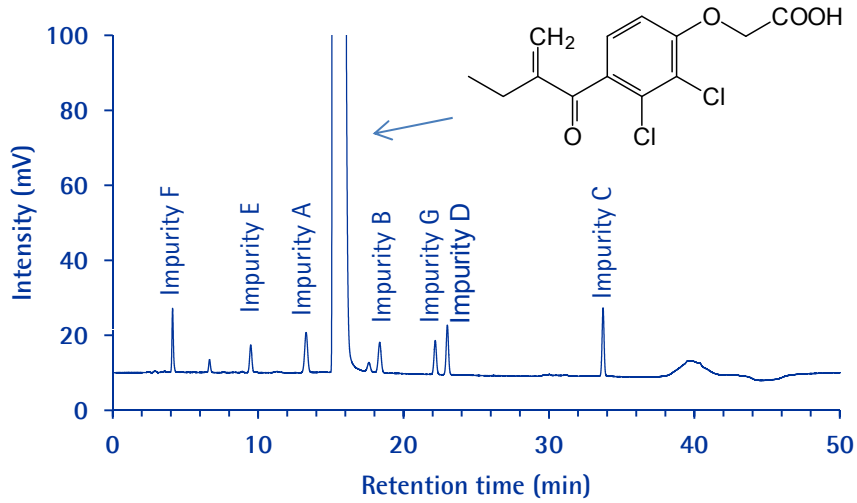
3. **Better overall resolution for more hydrophobic impurities**
   (whereas better overall resolution is attained for the less hydrophobic impurities on the particulate column)

Ethacrynic Acid and Related Impurities

Purospher® STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher® STAR RP-18 endcapped (5µm) Hibar® RT 250x4.6 mm	1.51456.0001
Injection:	20 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	A: Weigh 10 g of acetic acid and dilute to 1L with water. Adjust pH to 4.5 with dilute ammonia B: Methanol	
Gradient:	See Table	
Temperature:	50 °C	
Diluent:	Water and acetonitrile 65:45 (v/v)	
Sample:	Weigh 100 mg of ethacrynic acid in 100 mL volumetric flask. This gives a concentration of 1000 ppm. Add each impurity standard to get a 1 ppm level of impurities. Dilute with diluent.	
Pressure Drop:	83 Bar (1203 psi)	



Time (Min)	% A	% B
0.0	50	50
10.0	50	50
35.0	20	80
40.0	20	80
42.0	50	50
50.0	50	50

Chromatographic Data

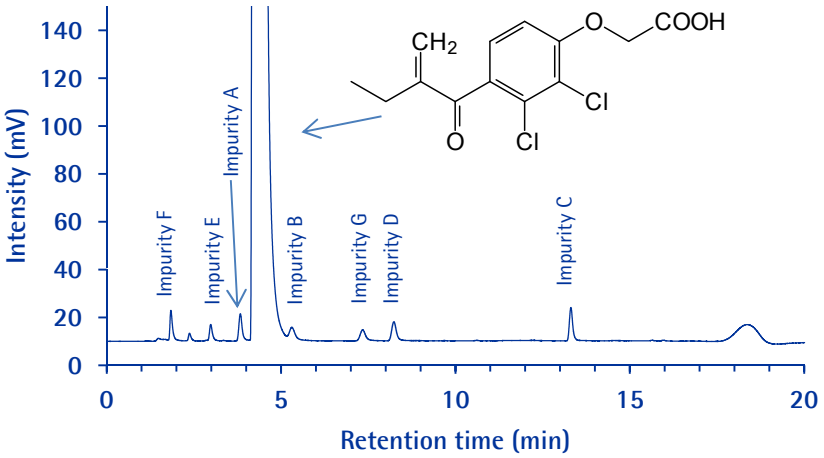
No.	Compound	Time (min)	T_{USP}	Theoretical Plates*
1	Impurity F	4.1	1.3	8745
2	Impurity E	9.5	1.0	17736
3	Impurity A	13.3	1.0	19539
4	Ethacrynic acid	15.2	4.3	16100
5	Impurity B	18.4	1.0	39284
6	Impurity G	22.2	1.0	81421
7	Impurity D	23.0	1.0	96857
8	Impurity C	33.7	1.0	275157

Ethacrynic Acid and Related Impurities

Chromolith® HighResolution RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm	1.52022.0001
Injection:	10 µL	
Detection:	UV 280 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	A: Weigh 10 g of acetic acid and dilute to 1L with water. Adjust pH to 4.5 with dilute ammonia. B: Methanol	
Gradient:	See Table	
Temperature:	45 °C	
Diluent:	Water and acetonitrile 65:45 (v/v)	
Sample:	Weigh 100 mg of ethacrynic acid in 100 mL volumetric flask. This gives a concentration of 1000 ppm. Add each impurity standard to get a 1 ppm level of impurities. Dilute with diluent.	
Pressure Drop:	71 to 57 Bar (1029 to 826 psi)	



Time (Min)	% A	% B
0.0	50	50
4.0	50	50
14.0	20	80
16.0	20	80
16.8	50	50
20.0	50	50

Chromatographic Data

No.	Compound	Time (min)	T_{USP}	Theoretical Plates*
1	Impurity F	1.8	1.6	3482
2	Impurity E	2.9	1.6	6983
3	Impurity A	3.8	1.3	8653
4	Ethacrynic acid	4.3	2.4	1380
5	Impurity B	5.3	1.2	6814
6	Impurity G	7.3	1.3	11073
7	Impurity D	8.2	1.3	19091
8	Impurity C	13.3	1.3	99494