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Application News

Gas Chromatograph Nexis™ GC-2030, AOC™-30i+20s U

Determination of Organic Impurities from Valproic Acid as per proposed USP monograph GC method

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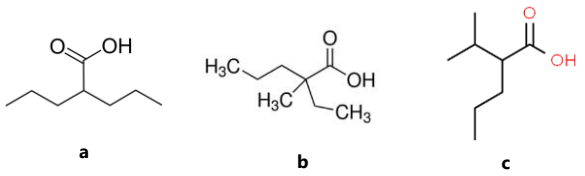
User Benefits

- Shimadzu Nexis GC-2030 can be effectively used for organic impurities test of valproic acid drug substance as per the proposed USP monograph GC method.
- The Nexis GC-2030 easily meets the acceptance criteria as per the proposed USP monograph for valproic acid

■ Introduction

Valproic acid, or valproate, is a fatty acid derivative and anticonvulsant originally used as a popular organic solvent in industry and pharmaceutical manufacturing for nearly a century, Currently it is a compound of interest in the field of oncology for its anti-proliferative effects and is the subject of many clinical trials in a variety of cancer types. It is on the World Health Organization's List of Essential Medicines and is available as a generic medication. The impurities such as valproic acid impurity B & valproic acid impurity K (Figure 1) originate through the manufacturing process of valproic acid.



a b c

Figure 1: Structure of a) Valproic acid ; b) Valproic acid compound K; c) Valproic acid compound B

Their presence should be controlled in finished valproic acid drug substance, this led United States Pharmacopeia (USP) to incorporate a gas chromatography procedure named "Organic Impurities" in the proposed new monograph for valproic acid below are the changes proposed in the upcoming monograph.

1. Revision of organic impurities test to add valproic acid related compound K with an acceptance criteria of NMT 0.15%, using USP G35 stationary phase.
2. Addition of USP valproic acid related compound K RS
3. Deletion of USP valproic acid related compound A RS

This application note demonstrates the determination of organic impurities from valproic acid as per proposed USP monograph GC method using Shimadzu's Nexis GC-2030 system. (Figure 2)

Figure 2: Nexis™ GC-2030 system

Nexis GC-2030, Key features

- ✓ Tool-free Column Installation
- ✓ One-Touch Inlet Maintenance
- ✓ Remote Operations and Monitoring
- ✓ Achieves Exceptional Reproducibility (AFC with CPU)
- ✓ Best-in-class sensitivity for most of the detectors

■ Experimental

Chromatographic conditions (Table 1), system suitability, standard and sample preparations were done in accordance with the proposed USP monograph for valproic acid. System suitability parameters were also checked as per the requirements of USP monograph. (Table 2, 3, 4 & 5)

Table 1: Instrument configuration and analytical conditions

GC System	**: Nexis GC-2030 with AOC-30i+20s U**		
Column	: SH-PolarD Cap. Column, 60 m, 0.32 mm, 0.50 µm (P/N: 227-36276-01)		
Injection Mode	: Split (5:1)		
Flow Control Mode	: Column flow		
Injector Port Temp.	: 220 °C		
Carrier Gas	: Helium		
Flow Rate	: 2.0 mL/min		
Injection Volume	: 1.0 µL		
Temp. Program	Ramp Rate (°C/min)	Temp. (°C)	Hold Time (min)
	-	100	5
	4	200	15
Detector	: Flame Ionization Detector (FID)		
Detector Temp.	: 220 °C		
Detector Gases	: Hydrogen, Air for flame & Helium for make up		
Air	: 200 mL/min		
Hydrogen	: 32 mL/min		
Helium (Make up)	: 24 mL/min		

System suitability, standard and sample preparations:

System suitability solution: 0.5 mg/mL of USP valproic acid RS, 0.05 mg/mL of USP valproic acid related compound B RS, and 0.05 mg/mL of USP valproic acid related compound K RS in n-heptane.

Standard solution: 0.005 mg/mL of USP valproic Acid RS in n-heptane.

Sensitivity solution: 0.0025 mg/mL of USP valproic Acid RS from standard solution in n-heptane.
(Further, 5-time diluted solution (0.0005mg/mL) was prepared & injected to showcase the sensitivity at even lower levels using Shimadzu GC-2030)

Sample solution: 5 mg/mL of valproic acid in n-heptane.

Results:

System suitability (SST) requirements

Relative Retention Time (RRT): The relative retention times for valproic acid related compound B, valproic acid related compound K & valproic acid are about 0.97, 0.98 & 1.0, respectively.

Table 2: RRTs of valproic acid impurities

Compound	RRT	
	Expected	Found
Valproic acid related compound B	0.97	0.97
Valproic acid related compound K	0.98	0.98

Resolution: Not less than (NLT) 1.5 between valproic acid related compound B and valproic acid related compound K, System suitability solution

Table 3: Resolution between valproic acid impurities

Compound	Resolution	
	Limit	Found
Valproic acid related compound K	NLT 1.5	2.3

Tailing factor: Not more than (NMT) 1.5 for valproic acid, Standard solution

Table 4: Tailing factor for valproic acid

Compound	Tailing Factor	
	Limit	Found
Valproic acid	NMT 1.5	1.0

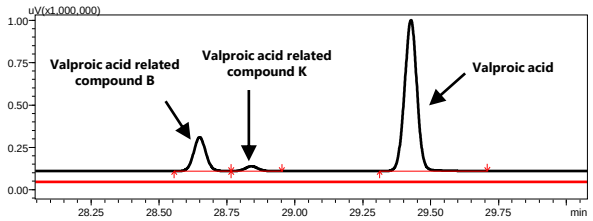
Signal-to-noise ratio (S/N) : NLT 10 for valproic acid, Standard solution

Table 5: S/N for valproic acid

Valproic acid-sensitivity solution	Signal-to-noise ratio	
	Expected	Found
0.5 ppm (Shimadzu)	NLT 10	19
2.5 ppm (USP)	NLT 10	181

The system suitability for organic impurities test passed as per criteria mentioned in proposed USP monograph.

Chromatograph of diluent blank & SST solution (Figure 3)



Black: SST solution ; Red: Diluent blank

Figure 3 Representative chromatograph for blank and SST solution

Sample results

Calculate the percentage of each impurity in the portion of valproic acid taken: Result = $(r_U / r_S) \times (C_S / C_U) \times (1/F)\ 100$

r_U = peak response of each impurity from the Sample
r_S = peak response of valproic acid from the Standard
C_S = concentration of USP valproic acid RS in the Standard solution (mg/mL)
C_U = concentration of valproic acid in the sample solution (mg/mL)
F= relative response factor

Table 6: Summary of content (%) of individual impurities in valproic acid sample replicates

Injection #	Unk.	Unk.	Unk.	B	K	VA
Injection-1	0.04	0.03	0.02	0.03	0.04	NA
Injection-2	0.04	0.03	0.02	0.02	0.04	NA
Injection-3	0.04	0.03	0.02	0.02	0.04	NA
Injection-4	0.04	0.03	0.02	0.03	0.04	NA
Injection-5	0.04	0.03	0.02	0.03	0.04	NA
Injection-6	0.04	0.03	0.02	0.02	0.04	NA

Unk.: Unknown ; B: Valproic acid related compound B; K: Valproic acid related compound K ; VA: Valproic acid

■ Conclusion

- This study successfully demonstrated the performance of Shimadzu Nexis GC-2030 system to determine the content of organic impurities in valproic acid sample as per the proposed USP monograph.
- The parameters for SST such as RRT, resolution, tailing factor and S/N meets the expected criteria.

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