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Accurate and reliable quantitation of *p*-toluenesulfonates genotoxic impurities in an active pharmaceutical ingredient by HPLC-UV

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Introduction

p-toluenesulfonic acid is frequently used in the manufacturing process as a counterion to salt formation.[1] *p*-toluenesulfonates (Figure 1) may be formed when the acid reacts with methanol, ethanol, or propanol used in the reaction pathway. These compounds are carcinogenic; therefore, they need to be accurately monitored and quantified both during the process and in the final control of the drug substance. The United States Food and Drug Administration (U.S. FDA) as well as the European Medicines Agency (EMA) have established a threshold of toxicological concern (TTC) of 1.5 µg/day for genotoxic impurities for long-term treatments with a drug product.[2,3] High performance liquid chromatography with UV detection (HPLC-UV) is the preferred analytical method for quantitation of impurities in a drug substance. Since genotoxic impurities must be detected at a lower level than other impurities, a very sensitive UV detector is required. Moreover, a wide linear range is desirable when reporting a genotoxic impurity as the relative integrated peak area of the active pharmaceutical ingredient (API).

In this work, an HPLC-UV method for the quantitation of four *p*-toluenesulfonates is presented. The determination of linearity, limit of detection (LOD), limit of quantification (LOQ), and recovery rates are shown.

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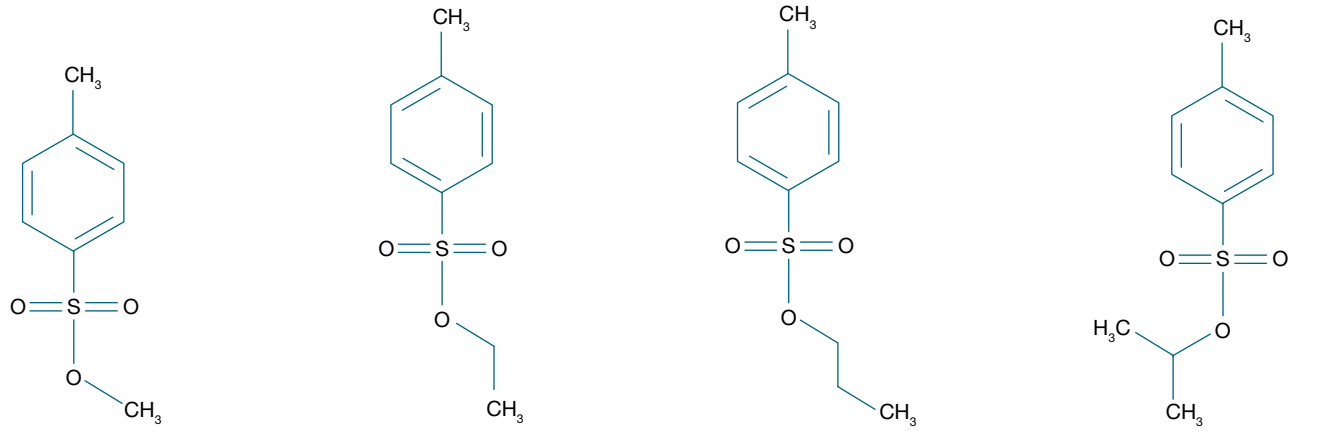

Methyl-*p*-toluenesulfonate **Ethyl-*p*-toluenesulfonate** **Propyl-*p*-toluenesulfonate** **Isopropyl-*p*-toluenesulfonate**

Figure 1. Chemical structures of the *p*-toluenesulfonates investigated in this study

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Acetonitrile Optima™ LC/MS grade (P/N A955-212)
- Fisher Scientific™ Methanol Optima™ LC/MS grade (P/N A456-212)
- Fisher Scientific™ Ammonium acetate LC/MS grade (P/N A114-50)
- Fisher Scientific™ Methyl-*p*-toluenesulfonate (P/N AAA1088130)
- Fisher Scientific™ Ethyl-*p*-toluenesulfonate (P/N AC147230500)
- Fisher Scientific™ Isopropyl-*p*-toluenesulfonate (P/N 13323651)
- Fisher Scientific™ Propyl-*p*-toluenesulfonate (P/N AC380600250)

An API sample (aprepitant) was purchased from a reputable vendor.

Equipment

- Vials (amber, 2 mL), Fisher Scientific (P/N 11545884)
- Snap Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 10547445)

Preparation of standards

Individual stock solutions of methyl-*p*-toluenesulfonate (methyl-TSF), ethyl-*p*-toluenesulfonate (ethyl-TSF), isopropyl-*p*-toluenesulfonate (isopropyl-TSF), and propyl-*p*-toluenesulfonate (propyl-TSF) were prepared in acetonitrile at a concentration of 1 mg/mL. A mixture containing all analytes with 10 µg/mL was prepared by diluting each stock solution with water/acetonitrile 50/50 (v/v). Based on this solution, calibration standards were prepared with concentrations of 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, and 2.5 µg/mL.

Additionally, a standard solution at 0.005 µg/mL was prepared for the determination of LOD.

Preparation of samples

A solution of 1 mg/mL of aprepitant sample was prepared in water/acetonitrile 50/50 (v/v) and filtered.

For the determination of recovery rates the aprepitant sample (1 mg/mL) was spiked with 0.01 µg/mL, 1 µg/mL and 2.5 µg/mL of the genotoxic impurities.

Instrumentation

A Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system was used for the analysis:

- Thermo Scientific™ Vanquish™ System Base Vanquish Horizon/Flex (P/N VH-S01-A)
- Thermo Scientific™ Vanquish™ Quaternary Pump F (P/N VF-P20-A)
- Thermo Scientific™ Vanquish™ Sampler FT (P/N VF-A10-A)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A)
- Thermo Scientific™ Vanquish™ Diode Array Detector FG (P/N VF-D11-A) with semi-micro flow cell, biocompatible, 2.5 µL (P/N 6083.0550)

Table 1. Acquisition method

Column:	Thermo Scientific™ Acclaim™ Polar Advantage II, 150 × 2.1 mm, 2.2 µm (P/N 071401)	
Mobile phase:	A: 15 mM ammonium acetate B: methanol	
Flow rate:	0.3 mL/min	
Gradient:	*Time [min]*	*% B*
	0	60
	4.5	60
	6.0	73
	21.0	73
	21.5	60
	35.0	60
Active pre-column heater temp.:	35 °C	
Column temp.:	35 °C (forced air mode, fan speed 5)	
Autosampler temp.:	4 °C	
UV wavelength:	225 nm	
3D scan:	190–280 nm	
UV data collection rate:	10 Hz	
UV response time:	0.5 s	
Injection volume:	10 µL	
Needle wash:	50% methanol	

Data processing and software

Data acquisition and processing were performed with the Thermo Scientific™ Chromeleon™ 7 Chromatography Data System (CDS) software.

Results and discussion

Calibration curves for all four analytes were obtained by triplicate injections of ten concentration levels (0.01 µg/mL–2.5 µg/mL). An excellent linearity could be achieved with correlation coefficients $R^2 > 0.9998$ for all four impurities (Figure 2).

The LOD and LOQ values were determined by diluting the standard mixture until a signal-to-noise ratio (S/N) close to 3 for LOD and 10 for LOQ was observed. The exact concentrations corresponding to S/N 3 and 10 were then calculated based on extrapolation from the measured values. Each sample was injected five times. Low LOQ values, in the order of 10 ng/mL, could be obtained (Table 2).

Recovery rates were estimated by spiking the aprepitant sample with 0.01 µg/mL (LOQ Level), 1 µg/mL (concentration limit based on TTC value) and 2.5 µg/mL (highest calibration level) with each genotoxic impurity. Excellent recovery rates were achieved on all spike levels with 90–99%. Only isopropyl-TSF shows slightly lower recovery on LOQ level with 73%, but is still acceptable (Table 3).

The wide dynamic range of the DAD FG detector allows relative quantitation of genotoxic impurities down to 0.02% relative area. Figure 3 shows the chromatogram of an aprepitant sample spiked with the genotoxic impurities at LOQ level. The signal response of the impurities are approximately 10,000 times lower than for the API peak.

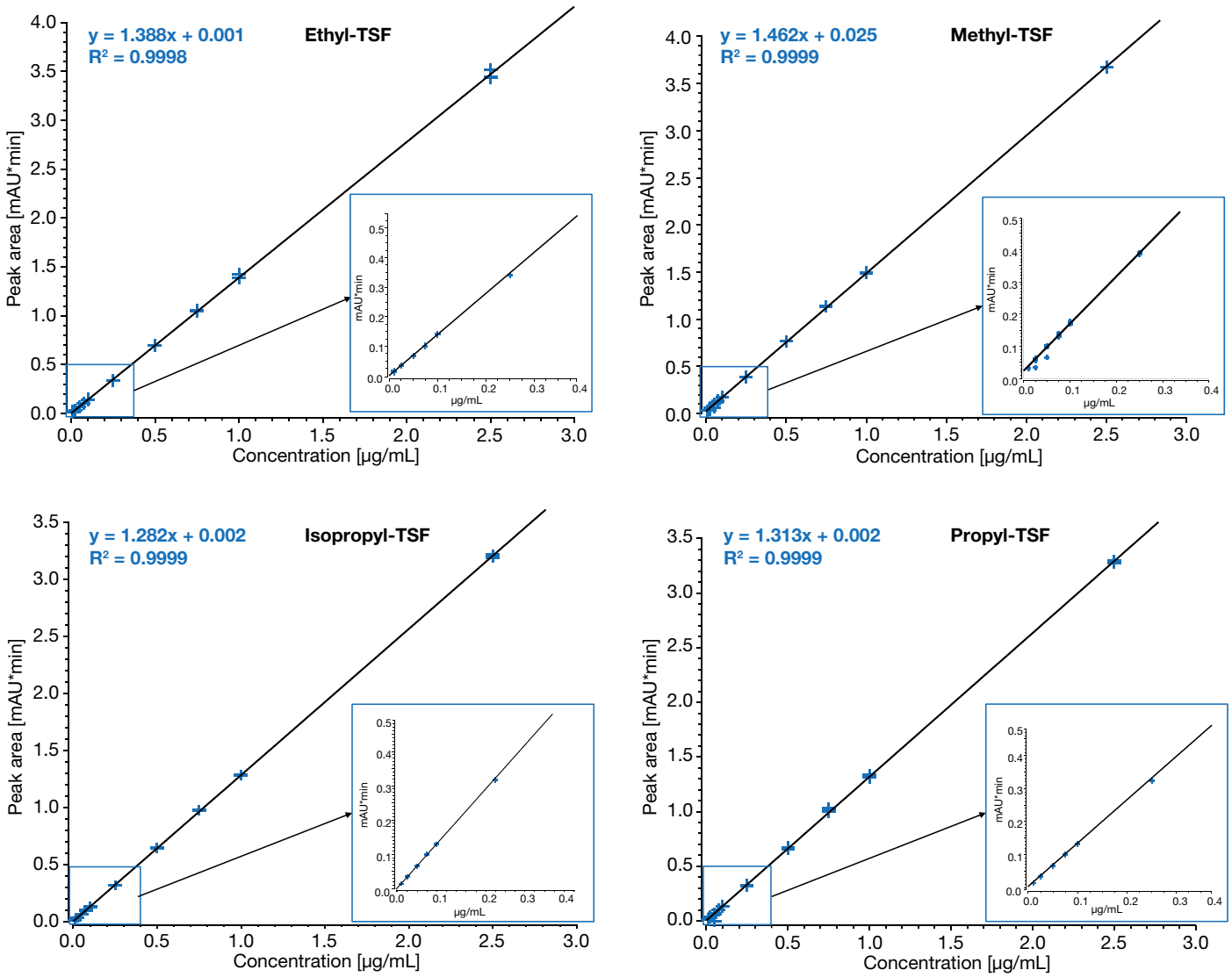

Figure 2. UV calibration curves of ethyl-TSF, methyl-TSF, isopropyl-TSF, and propyl-TSF over the concentration range of 0.01 µg/mL to 2.5 µg/mL, with zoomed view into the lower concentration range

Table 2. LOD and LOQ values with standard deviation (SD)

Compound	Methyl-TSF	Ethyl-TSF	Isopropyl-TSF	Propyl-TSF
LOD [ng/mL] ± SD	3.3 ± 0.7	3.5 ± 1.1	4.0 ± 0.8	4.1 ± 0.7
LOQ [ng/mL] ± SD	9.4 ± 1.9	9.5 ± 1.8	10.8 ± 2.7	10.7 ± 2.6

Table 3. Recovery rates on different spike levels

Compound	Methyl-TSF	Ethyl-TSF	Isopropyl-TSF	Propyl-TSF
Recovery [%] at 0.01 µg/mL	93	93	73	90
Recovery [%] at 1 µg/mL	98	98	92	98
Recovery [%] at 2.5 µg/mL	99	99	93	99

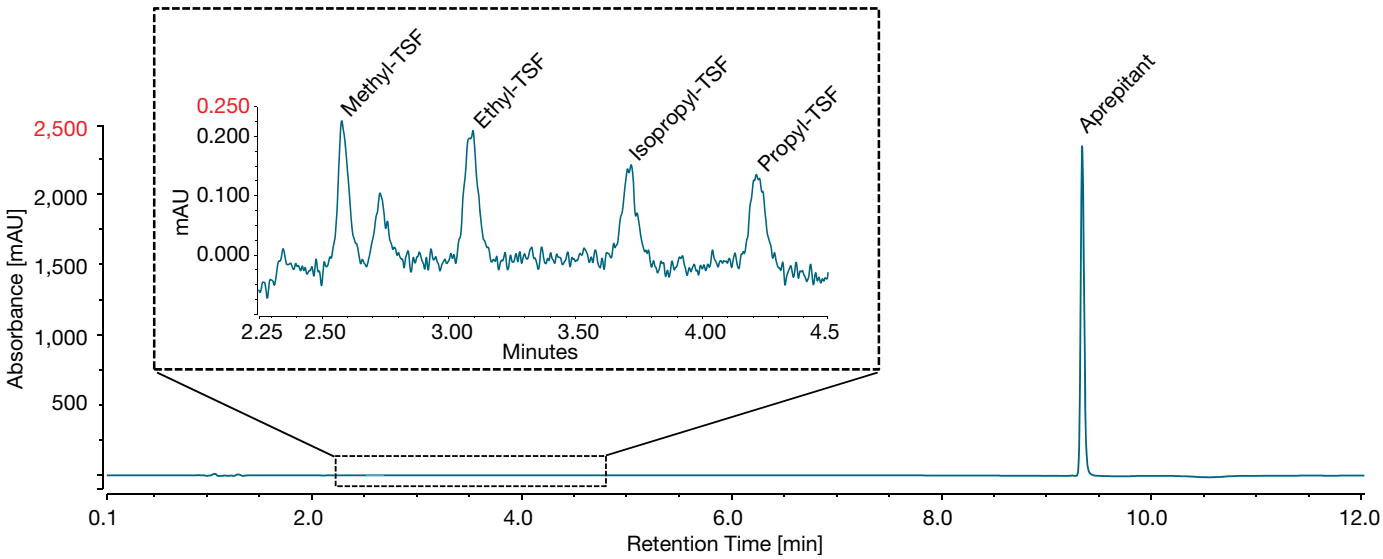

Figure 3. Chromatogram of an aprepitant sample spiked with 0.01 µg/mL (LOQ level) of genotoxic impurities

Conclusion

The method proved to be very sensitive for the determination of four genotoxic *p*-toluenesulfonate impurities with LOD values less than 5 ng/mL and LOQ values less than 13.5 ng/mL. The excellent dynamic range and sensitivity of the Vanquish DAD FG allows accurate quantification over a wide concentration range of 0.01 µg/mL to 2.5 µg/mL. In addition, high recovery rates (90–99%) demonstrate the ability of the method to reliably quantify genotoxic impurities in active pharmaceutical ingredients.

References

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