A Sensitive and Selective LC-UV-MS Method for Determining a Genotoxic Impurity in a Drug Sample

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ABSTRACT

Genotoxic impurities and potential gentotoxic impurities are reactive compounds that can induce mutations by reacting with DNA. They are a major concern for the pharmaceutical industry and regulatory agencies as their presence in active pharmaceutical ingredients (API) can potentially cause cancer in the patient. Many potential genotoxic impurities however are useful in chemical synthesis and it is almost impossible to eliminate them all during API synthesis. The genotoxic impurities identified as potential contaminant of the drug must thus be monitored and accurately quantified according to rules which are stricter than for other impurities.
The method we developed demonstrates the ability to monitor and quantify the genotoxic impurity methyl-*p*-toluenesulfonate along with three related impurities in the drug substance aprepitant by an LC-UV-MS approach using the MS data for mass based identification and the UV data for quantification. To unequivocally confirm the presence of methyl-*p*-toluenesulfonate in the sample, a spike-in experiment was performed.

INTRODUCTION

The analysis of process- and product-related impurities is an essential step throughout the lifecycle of a drug. High performance liquid chromatography (HPLC) with ultraviolet light (UV) detection is the most commonly applied technique to assess the purity of an active pharmaceutical ingredient. Identification is typically assessed based on retention time. For this purpose, single standards have to be run separately. During the early drug development stages, standards relating to impurities are often not available, and thus the identity of peaks cannot be easily assigned.

Genotoxic impurities are regulated by the United States Food and Drug Administration (USFDA) as well as the European Medicines Agency (EMEA). They have established a threshold of toxicological concern (TTC) of 1.5 µg/day for long-term treatment with the drug product [1, 2]. Additionally, the international conference on harmonization (ICH) M7 suggests a staged TTC based on the duration of drug exposure as described in Table 1 [3].

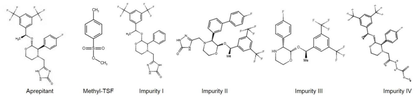
Table 1. Acceptable daily intakes for an individual impurity

	Duration of exposure			
	< 1 month	> 1 – 12 month	> 1 – 10 years	> 10 years
Acceptable daily intake for an individual impurity [µg/day]	120	20	10	1.5

P-toluenesulfonates are examples of genotoxic impurities. They can occur as by-products of the drug synthesis where *p*-toluenesulfonic acid reacts with alcohols such as methanol, ethanol or isopropanol. Since *p*-toluenesulfonic acid is frequently used as a counterion for salt formation, *p*-toluenesulfonates are fairly common as genotoxic impurities [4]. The identification of a *p*-toluenesulfonate is relatively straightforward as standards are readily available. However, it is practical and desirable that HPLC methods, during the early stage of drug development, are capable of identifying genotoxic as well as other related impurities in a single run without the need for separate injections of standards.

The aim of this study was to develop a method for monitoring and quantifying methyl-*p*-toluenesulfonate along with other related impurities in a drug substance. The API chosen for this example is aprepitant. Aprepitant is an antiemetic usually administered for the prevention of nausea and vomiting after chemotherapy. Contamination of aprepitant by genotoxic methyl-*p*-toluenesulfonate (methyl-TSF) may occur, since *p*-toluenesulfonic acid and methanol are used in different steps of the synthesis [4,5].

Figure 1: Chemical structures of the API aprepitant and related impurities investigated here



Annotation on chemical names of impurities:
Impurity I = Defluoro Aprepitant;
Impurity II = 4-Defluoro-4-(p-fluorophenyl)aprepitant;
Impurity III = Des-1,2,4-triazol-3-one-5-methyl –aprepitant;
Impurity IV = N-(Destriazolonomethyl) N-(Methylcarboxyacetamidohydrazono)aprepitant)

INSTRUMENTATION AND METHODS

A Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system equipped with an ISQ™ EM

Single Quadrupole Mass Spectrometer was used for the analysis.

- System Base Vanquish Horizon/ Flex (VH-S01-A)
- Quaternary Pump F (VF-P20-A)
- Sampler FT (VF-A10-A)
- Column Compartment H (VH-C10-A)
- Diode Array Detector F (VF-D11-A) with semi-micro flow cell, 2.5 µL (6083.0550)
- ISQ EM mass spectrometer (ISQEM-ESI)

Table 2. Chromatographic Conditions

Parameter	value	
Column	Thermo Scientific™ Acclaim™ Polar Advantage II (150 x 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
Column Temp	35 °C (with Active pre-column heater at 35° C)	
Sampler Temp	4 °C	
UV	λ = 225 nm, data collection rate = 10 Hz, response time = 0.5 s 3D Scan 190-280 nm	
Injection vol.	10 µL	

Table 3. MS Conditions

Source Parameters	Autospray HESI source settings for 0.3 mL/min flow rate
Sheath gas pressure	35.8 psig
Aux gas pressure	4.0 psig
Sweep gas pressure	0.5 psig
Vaporizer temperature	172 °C
Ion transfer tube temperature	250 °C
Source voltage	3000 V
Method type	Full Scan + six targeted SIM Scans
Ion polarity	positive
Mass range	*m/z* 100-650
SIM width	0.5
Dwell Time	0.1 s
Source CID voltage	10 V

Data Analysis

The analysis was performed with the Thermo Scientific™ Chromeleon™ 7.2.9 Chromatography Data System (CDS) software, which fully integrates the ISQ EM mass detector enabling instrument control, data acquisition and data processing on a single software platform.

Sample preparation

A stock solution of methyl-*p*-toluenesulfonate was prepared in acetonitrile at a concentration of 1 mg/mL.

Aprepitant I sample contained methyl-TSF as an impurity and was used for the impurity screening experiments and quantitation.

Aprepitant II sample was found to be a methyl-TSF free sample and was therefore used for the determination of recovery rates by spiking methyl-TSF into the aprepitant II sample (1mg/mL).

RESULTS

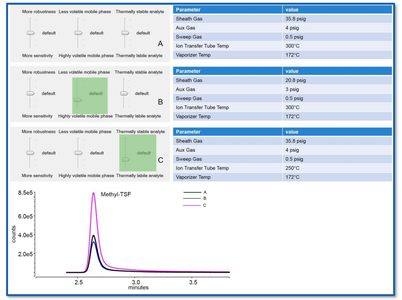
Adjustment of ion source parameters of the mass detector

Automated adjustment of the ion source parameters of the ISQ EM mass detector can be carried out with a feature implemented in Chromeleon CDS. The software allows for an automatic optimization of source CID voltage by using sequence custom variables in the injection list. The signal-to-noise (S/N) ratio was compared to determine the best CID voltage for methyl-TSF. A CID voltage of 10 V was found to provide a signal with maximum intensity.

Moreover, Autospray intelligent ion source settings allow for an easy adjustment of the gas and temperature settings of the ion source based on the flow rate used in the chromatographic method. Three aspects are given special consideration: robustness/ sensitivity, volatility of mobile phase and thermal stability of analytes.

Figure 2 demonstrates a selection of Autospray ion source settings and the effect on gas and temperature values when using different slider positions.

Figure 2: MS ion source parameter adjustment with Autospray intelligent ion source settings concept; A- Default position; B- varied position on volatility of mobile phase, C- varied position on thermal analyte stability



The overlay of the extracted ion chromatograms (XICs) as shown in Figure 2 helps to identify the optimal settings providing maximum signal intensity. Here, the highest signal intensity was achieved using the settings of Figure 2-C.

Impurity Screening

Genotoxic impurity, methyl-TSF, and several known non-genotoxic impurities of aprepitant [6,7] were investigated in the study and qualitatively monitored (Table 4). Chemical structures of the analytes are presented in Figure 1. Data were acquired in SIM scan mode to allow for a more sensitive detection. Methyl-TSF exclusively forms the ammonium adduct $[M+NH4]^+$, when ammonium acetate as aqueous mobile phase is used, while the API and its non-genotoxic related impurities mainly form $[M+H]^+$ ions.

Table 4: Aprepitant and five related impurities with their detected ion species and related *m/z* values targeted in the detection via SIM scans.

Compound	Aprepitant	Methyl-TSF	Impurity I	Impurity II	Impurity III	Impurity IV
SIM Scan *m/z*	535.2	204.1	517.2	611.2	438.1	567.2
Ion species	$[M+H]^+$	$[M+NH4]^+$	$[M+H]^+$	$[M+H]^+$	$[M+H]^+$	$[M+H]^+$

LC-UV-MS analysis of the API sample (aprepitant I) resulted in the UV chromatogram as shown in Figure 3 containing several low abundant peaks next to the major peak representing the API. For MS detection, SIM scans were performed targeting masses as listed in Table 4 to verify the presence of expected impurities (Figure 4). Four impurities were confirmed by SIM scans including the genotoxic one methyl -TSF. For impurity II; the SIM scan chromatogram provides only a very weak signal with a strong baseline effect and the UV trace did not show any peak at the retention time of the MS detected weak signals. Thus, the presence of impurity II in the sample could not be confirmed. Chromeleon CDS allows to account for the time delay between the UV and MS signals, which greatly facilitates mass assignment of peaks in the UV chromatogram. To monitor possible unexpected impurities, the applied MS method contained also full MS scans in addition to the target SIM Scans. Two additional impurities with *m/z* 105.4 at retention time (RT) 2.1 min and *m/z* 593.1 at RT 11.7 min could be assigned, however no further investigation on the identity of these impurities was performed.

Figure 3: UV chromatogram acquired at 225 nm of aprepitant I sample with peak assignments obtained from MS data; grey boxes: zoom into baseline of API and related impurities; only peaks with > 0.03% relative area are considered

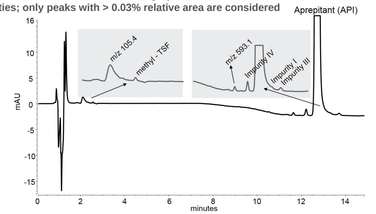
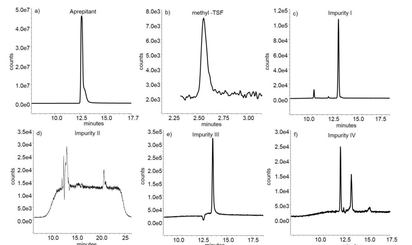


Figure 4: SIM scan chromatograms of aprepitant I (a) and related impurities according to Table 4 (b-f).



To confirm the presence of methyl-TSF in the sample, the compound was spiked and analyzed under the same condition (Figure 5). A calibration curve was obtained over a concentration range of 0.01 µg/mL-2.5 µg/mL achieving excellent linearity.

Figure 5: Overlaid UV chromatograms of spiked API sample (blue) and unspiked sample (black)

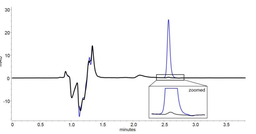
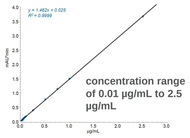


Figure 6: Calibration curve of Methyl-TSF based on UV detection



Quantitation of methyl-TSF in the sample using UV detection

Quantitative analysis was carried out by triplicate injection of the API sample aprepitant I. As shown in Table 5 the calculated amount corrected by the recovery rate (estimated by spiking methyl-TSF into aprepitant sample II) resulted in 0.010 µg/mL with a relative standard deviation (RSD) of 5.05%. As a result, the genotoxic impurity detected in the sample is far below the acceptable concentration limit based on the TTC value (0.96 µg/mL) and close to the LOQ limit (0.0094 µg/mL) of the method.

Table 5. Quantitation result of methyl-*p*-toluenesulfonate in aprepitant I sample

Measured amount [µg/mL]	Recovery [%]	Calculated amount [µg/mL]	Calculated amount RSD [%]
0.009	93	0.010	5.05

CONCLUSIONS

- The combination of UV and mass detection on a single quadrupole mass detector is a powerful tool for monitoring, detection and quantification impurities in APIs in early stage of development.
- With a 10-fold lower LOQ value compared to the acceptable concentration limit based on the TTC value of 960 ng/mL, the UV method proved to be very sensitive to the determination of methyl-*p*-toluenesulfonate.
- In addition, excellent linearity and recovery rates were demonstrated for the genotoxic impurity methyl-TSF.
- The Autospray intelligent source settings concept allows a non-experienced MS user an easy and fast adjustment of the MS ion source parameters.

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