Analysis of Potential Genotoxic Impurities in the Drug Development Process Using GC-MS/MS

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Overview

- Three potential genotoxic impurities (PGIs) with nitro-aromatic rings need to be characterized and quantified in the active pharmaceutical ingredients (APIs)
- LC-MS characterization of PGIs with non-polar functional groups in drug development is challenging due to poor atmospheric pressure ionization efficiency, such as ESI, APCI and APPI
- In the current study, a novel GC-MS/MS method was developed for quantitative analysis of PGI with halogenated nitro-aromatics in API matrices
- The limit of detection (LOD) of PGIs in the drug matrices can achieve < 0.2 ppm (µg/g), satisfying the regulated quantitative requirement for pharmaceutical impurities

Introduction

- The goal of this study was to develop a sensitive method for quantitation of the three nitro-aromatic PGIs at less than 1 ppm (µg/g) in two different APIs to meet the regulatory and compliance requirement.
- Characterization of trace potential genotoxic impurities (PGIs) in the drug development process is commonly performed using LC-MS. Although LC-MS has broad applicability, it shows big limitations analyzing impurities poorly ionized, such as the nitro-aromatics, one type of PGIs required characterization and quantitation in the active pharmaceutical ingredients (APIs).
- GC-MS is a complementary analytical technique, and is more advantageous when analyzing volatile and non-polar organic compounds. In the current work, GC-MS/MS was employed as the quantitative tool as LC-MS/MS demonstrated poor sensitivity due to the incapability of the three compounds with electro spray ionization. With good solubility in ACN, the target compound solution was directly injected to GC-MS. The EI full scan spectrum provided the characteristic information for target compound confirmation, enabling further quantitation.
- A quantitative GC-MS based MRM method was developed to analyze three halogenated nitro-aromatic PGI targets within the API medium. The GC-MS/MS method employing MRM mode exhibited excellent sensitivity and robustness relative to LC-MS method. Trace PGIs in APIs (< 0.2 ppm) can be easily detected.
- This GC-MS based MRM method for quantitation of the three nitro-aromatic PGIs meets the regulated sensitivity requirement of pharmaceutical impurity analysis (< 1ppm); GC-MS/MS is an alternative analytical tool of LC-MS for detection of trace PGIs in the drug development process

Method

Samples

- Standards: three halogenated PGI compounds (in dry powder), labeled as PGI 1, PGI 2, and PGI 3
- Matrices: two active pharmaceutical ingredients (in dry powder), labeled as API 1, and API 2

Sample preparation

- PGI 1-3 were dissolved in acetonitrile as the standard mix stock
- Quantitation working solutions were prepared in pure acetonitrile for the quantitative GC-MS/MS method development, the concentration is from 2 – 1000 µg/L in acetonitrile
- API 1 and API 2 were dissolved in high concentration in acetonitrile (10 mg/mL) as the matrix solutions
- The PGI stock mix was spiked into API 1 and API 2 with known concentration respectively to mimic the contaminated API solutions

Instrument conditions

- **Instrument:** Bruker Scion TQ GC-MS/MS coupled to Bruker 436 GC system
- **GC column:** Bruker BR-5ms, 30 m × 0.25 mm ID and 0.25 µm film thickness
- **Carrier Gas:** He, 1mL/min
- **Oven Temp:** 50 °C (1 min), 15 °C /min to 300 °C (1 min), 30 °C /min to 315 °C (15min)
- **Injector:** Pulsed Splitless (40 psi, 0.3 min); 280°C
- **Injection volume:** 1 µL
- **EI source Temp:** 260 ◦ C
- **Transfer line Temp:** 280 ◦ C
- **Emission Current:** 80 µA
- **Electron voltage:** 70eV
- **Q2 CID Gas Pressure:** 1.5mTorr

Figure 1. Bruker Scion TQ GC-MS/MS system, coupled to Bruker 436 GC (left with touch screen), and Bruker 8400 Auto sampler

Results

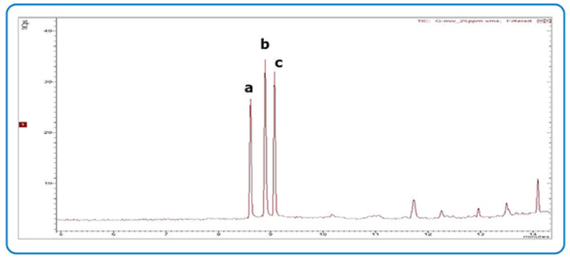

Figure 2. Full scan GC-MS spectrum of a mix of PGI 1 (a), PGI 2 (b), PGI 3 (c) in acetonitrile (25 mg/L); a nice base line separation of three PGI compounds was achieved using the optimized GC-MS condition.

Compound	RT (min)	RT window (min)	MRM1 (eV)	MRM 2 (ev)	Scan time (ms)
PGI 1	10.85	0.5	184 > 138 (15 eV)	184 > 110 (25 eV)	200
PGI 2	11.17	0.5	172 > 126 (20 eV)	172 > 90 (22 eV)	200
PGI 3	11.39	0.5	283 > 225 (20 eV)	283 > 126 (20 eV)	200

Table 1. The optimized MRM transitions of the three PGI compounds in the current study; the MRM method was extended as a quantitative MRM method for PGI quantitation in the API matrices

Matrix	Linearity coefficient (r^2) (2 – 1000 µg/L in matrix solution)		
	PGI 1	PGI 2	PGI 3
Acetonitrile	0.998	1.000	0.999
API 1 (10 mg/mL in ACN)	0.999	0.998	0.999
API 2 (10 mg/mL in ACN)	0.990	0.999	1.000

Table 2. Quantitation of three PGI components in different matrices: pure acetonitrile, API 1 and 2 solution reconstituted in ACN (10 mg/mL); the calibration dynamic range is from 2 – 1000 µg/L in the matrix solutions; if convert the quantitation unit to PGI vs. API, the dynamic range of PGI quantitation in API 1 and API 2 solution is 0.2 – 100 µg/mg (ppm).

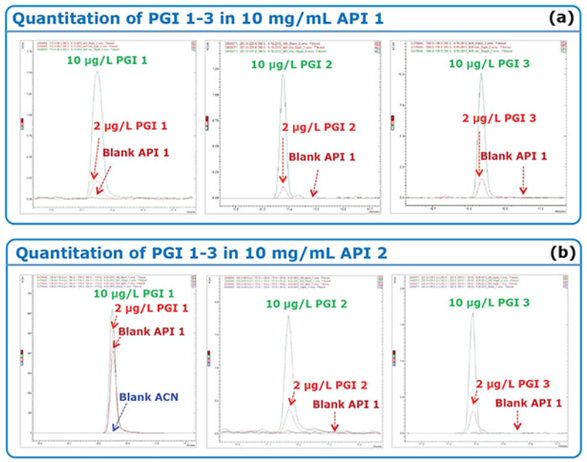

Figure 3. Quantitation of the three PGI compounds in the 10 mg/mL (a) API 1 in ACN; (b) API 2 in ACN; the limit of quantitation (LOQ) of PGI 1-3 is 0.2 mg/g in the 10 mg/mL API 1 solution (Figure 3a); the LOQ of PGI 2 and PGI 3 in the 10 mg/mL API 2 solution is 0.2 mg/g (Figure 3b); Figure 3b also shows the blank API 2 matrix was contaminated by trace PGI 1, therefor detection sensitivity of PGI 1 in API 2 is ~ 1 mg/g in the presence of 10 mg/mL API 2 in ACN solution

Conclusions

- A novel GC-MS/MS method was developed for quantitative analysis of 3 PGI compounds with halogenated nitro-aromatics in two different API matrices
- The GC-MS/MS method achieves base-line separation of the three PGI compounds with nice peak shapes in the chromatography
- The GC-MS/MS based MRM method with a calibration range from 2 – 1000 µg/L was developed to quantify the three PGI in the high background API matrix (10 mg/mL in pure ACN)
- The limit of detection (LOD) of PGIs in the API matrices can achieve the sensitivity ~ 0.2 ppm (µg/g)
- This GC-MS/MS method is a sensitivity method for PGI quantitation, satisfying the regulated quantitative requirement for pharmaceutical impurities