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Highly sensitive quantitative estimation of genotoxic impurities from API and drug formulation using LC/MS/MS

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Introduction

The toxicological assessment of Genotoxic Impurities (GTI) and the determination of acceptable limits for such impurities in Active Pharmaceutical Ingredients (API) is a difficult issue. As per European Medicines Agency (EMEA) guidance, a Threshold of Toxicological Concern (TTC) value of 1.5 µg/day intake of a genotoxic impurity is considered to be acceptable for most pharmaceuticals[1].
Dronedarone is a drug mainly used for indications of cardiac arrhythmias. GTI of this drug has been quantitated here. Method has been optimized for simultaneous analysis of DRN-IA {2-n-butyl-3-[4-(3-di-n-butylamino-propoxy)benzoyl]-5-nitro benzofuran}, DRN-IB {5-amino-3-[4-(3-di-n-butylamino-propoxy)benzoyl}-2-n-butyl benzofuran} and BHBNB {2-n-butyl-3-(4-hydroxy benzoyl)-5-nitro benzofuran}. Structures of Dronedarone and its GTI are shown in Figure 1.
As literature references available on GTI analysis are minimal, the feature of automatic MRM optimisation in LCMS-8040 makes method development process less tedious. In addition, the lowest dwell time and pause time and ultrafast polarity switching of LCMS-8040 ensures uncompromised and high sensitive quantitation.

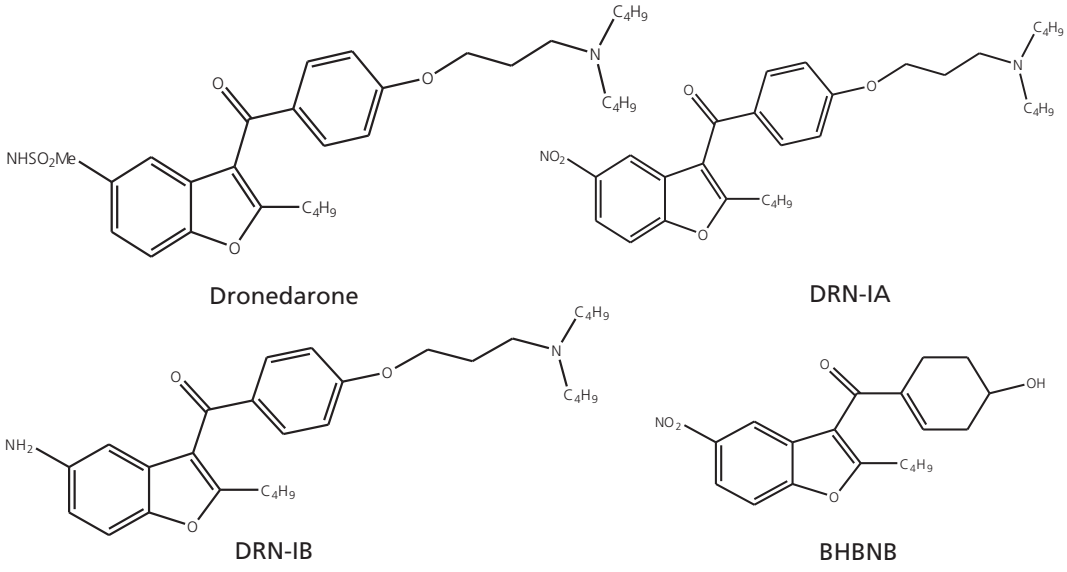

Figure 1. Structures of Dronedarone and its GTI

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Method of Analysis

Sample Preparation

- **Preparation of DRN-IA and DRN-IB and BHBNB stock solutions**
  20 mg of each impurity standard was weighed separately and dissolved in 20 mL of methanol to prepare stock solutions of each standard.

- **Preparation of calibration levels**
  GTI mix standards (DRN-IA, DRN-IB and BHBNB) at concentration levels of 0.5 ppb, 1 ppb, 5 ppb, 10 ppb, 40 ppb, 50 ppb and 100 ppb were prepared in methanol using stock solutions of all the three standards.

- **Preparation of blank sample**
  400 mg of Dronedarone powder sample was weighed and mixed with 20 mL of methanol. Mixture was sonicated to dissolve sample completely.

- **Preparation of spiked (at 12 ppb level) sample**
  400 mg of sample was weighed and spiked with 60 µL of 1 ppm stock solution. Solution was then mixed with 20 mL of methanol. Mixture was sonicated to dissolve sample completely.

LC/MS/MS Analytical Conditions

Analysis was performed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera coupled with LCMS-8040 triple quadrupole system (Shimadzu Corporation, Japan), shown in Figure 2. Limit of GTI for Dronedarone is 2 mg/kg. However, general dosage of Dronedarone is 400 mg, hence, limit for GTI is 0.8 µg/400 mg. This when reconstituted in 20 mL system, gives an effective concentration of 40 ppb. For analytical method development it is desirable to have LOQ at least 30 % of limit value, which in this case corresponds to 12 ppb. The developed method gives provision for measuring GTI at much lower level. However, recovery studies have been done at 12 ppb level.

Figure 2. Nexera with LCMS-8040 triple quadrupole system by Shimadzu

Below mentioned table shows the analytical conditions used for analysis of GTI.

Table 1. LC/MS/MS analytical conditions

• Column	: Shim-pack XR-ODS II (75 mm L x 3 mm I.D.; 2.2 µm)
• Mobile phase	: A: 0.1% formic acid in water B: acetonitrile
• Flow rate	: 0.3 mL/min
• Oven temperature	: 40 °C
• Gradient program (B%)	: 0.0–2.0 min → 35 (%); 2.0–2.1 min → 35-40 (%); 2.1–7.0 min → 40-60 (%); 7.0–8.0 min → 60-100 (%); 8.0–10.0 min → 100 (%); 10.0–10.01 min → 100-35 (%); 10.01–13.0 min → 35 (%)
• Injection volume	: 1 µL
• MS interface	: Electro Spray Ionization (ESI)
• MS analysis mode	: MRM
• Polarity	: Positive and negative
• MS gas flow	: Nebulizing gas 2 L/min; Drying gas 15 L/min
• MS temperature	: Desolvation line 250 °C; Heat block 400 °C

Note: Flow Control Valve (FCV) was used for the analysis to divert HPLC flow towards waste during elution of Dronedarone so as to prevent contamination of Mass Spectrometer.

Results

LC/MS/MS analysis

LC/MS/MS method was developed for simultaneous quantitation of GTI mix standards. MRM transitions used for all GTI are given in Table 2. No peak was seen in diluent (methanol) at the retention times of GTI for selected MRM transitions which confirms the absence of any interference from diluent (shown in Figure 3). MRM chromatogram of GTI mix standard at 5 ppb level is shown in Figure 4. Linearity studies were carried out using external standard calibration method. Calibration graphs of each GTI are shown in Figure 5. LOQ was determined for each GTI based on the following criteria – **(1)** % RSD for area < 15 %, **(2)** % Accuracy between 80-120 % and **(3)** Signal to noise ratio (S/N) > 10. LOQ of 0.5 ppb was achieved for DRN-IB and BHBNB whereas 1 ppb was achieved for DRN-IA. Results of accuracy and repeatability for all GTI are given in Table 3.

Table 2: MRM transitions selected for all GTI

Name of GTI	MRM transition	Retention time (min)	Mode of ionization
DRN-IB	479.15>170.15	1.83	Positive ESI
DRN-IA	509.10>114.10	5.85	Positive ESI
BHBNB	338.20>244.05	8.77	Negative ESI

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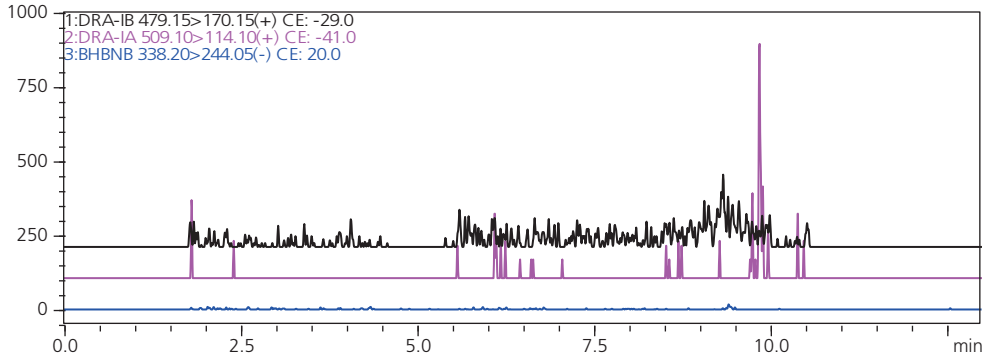

Figure 3. MRM chromatogram of diluent (methanol)

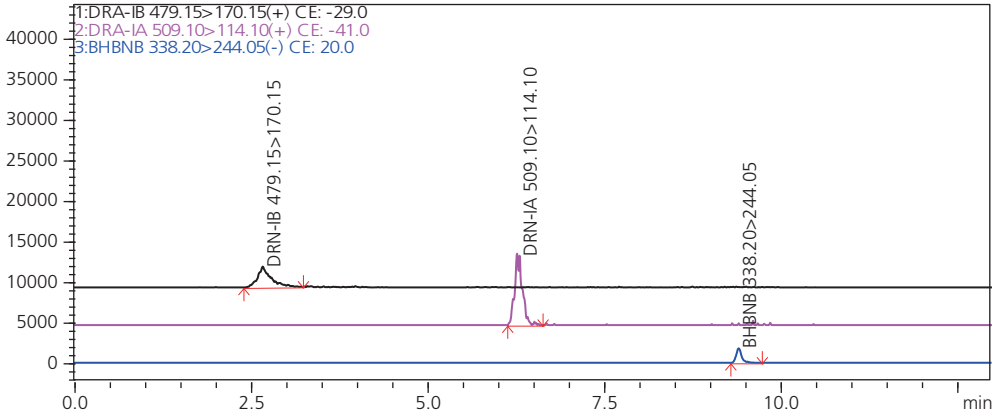

Figure 4. MRM chromatogram of GTI mix standard at 5 ppb level

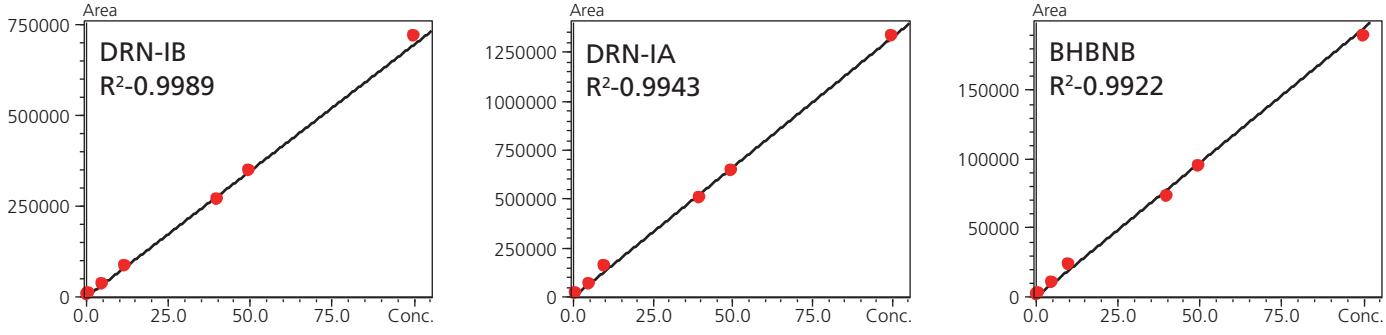

Figure 5. Calibration graphs for GTI

Table 3: Results of accuracy and repeatability for all GTI

Sr. No.	Name of GTI	Standard concentration (ppb)	Calculated concentration from calibration graph (ppb) (n=6)	% Accuracy (n=6)	% RSD for area counts (n=6)
1	DRN-IB	0.5	0.492	98.40	9.50
		1	1.044	104.40	6.62
		5	4.961	99.22	3.10
		12	12.014	100.12	2.97
		40	38.360	95.90	1.17
		50	49.913	99.83	1.08
		100	103.071	103.07	0.86
2	DRN-IA	1	0.994	99.40	5.02
		5	4.916	98.32	2.82
		12	11.596	96.63	2.43
		40	37.631	94.08	1.27
		50	48.605	97.21	1.40
		100	100.138	100.14	0.99
3	BHBNB	0.5	0.486	97.20	4.88
		1	1.062	106.20	6.97
		5	4.912	98.24	2.16
		12	11.907	99.23	1.31
		40	37.378	93.45	0.37
		50	48.518	97.04	0.43
		100	96.747	96.75	0.91

Recovery studies

For recovery studies, samples were prepared as described previously. MRM chromatogram of blank and spiked samples are shown in Figures 6 and 7 respectively. Results of recovery studies have been shown in Table 4. Recovery could not be calculated for DRN-IB as blank sample showed higher concentration than spiked concentration.

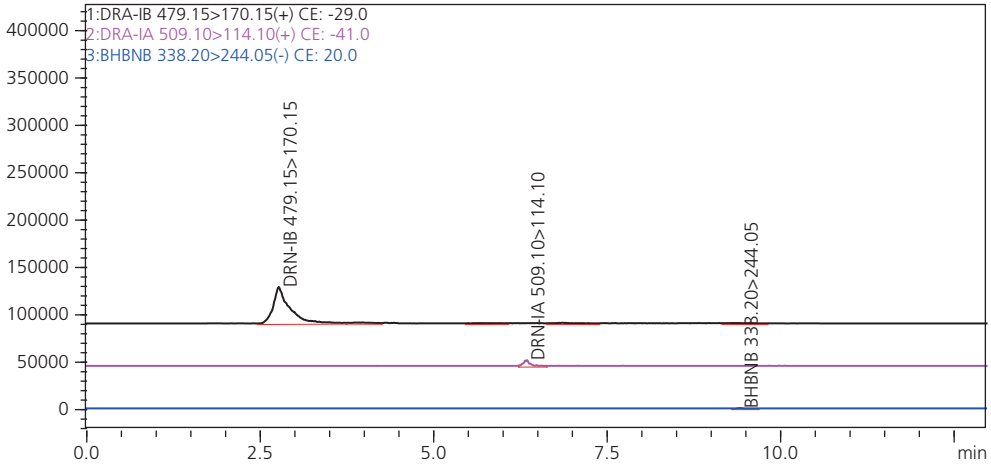

Figure 6. MRM chromatogram of blank sample

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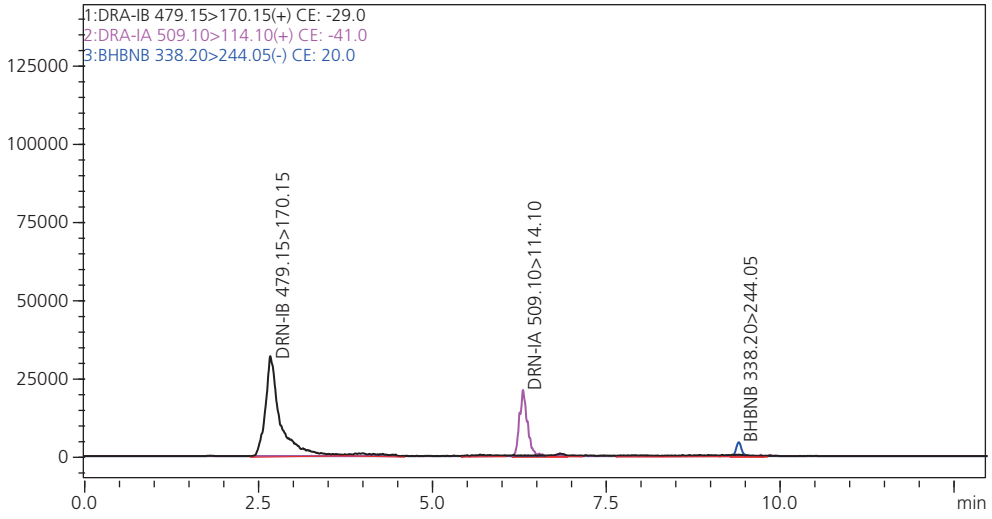

Figure 7. MRM chromatogram of spiked sample

Table 4. Results of the recovery studies

Name of Impurity	Concentration of GTI mix standard spiked in blank sample (ppb)	Average concentration obtained from calibration graph for blank sample (ppb) (A) (n=3)	Average concentration obtained from calibration graph for spiked sample (ppb) (B) (n=3)	% Recovery = (B-A)/ 12 * 100
DRN-IB	12	94.210	NA	NA
DRN-IA	12	3.279	12.840	79.678
BHBNB	12	1.241	12.723	95.689

Conclusion

- A highly sensitive method was developed for analysis of GTI of Dronedarone.
- Ultra high sensitivity, ultra fast polarity switching (UFswitching) enabled sensitive, selective, accurate and reproducible analysis of GTI from Dronedarone powder sample.

References

[1] Guideline on The Limits of Genotoxic Impurities, (2006), European Medicines Agency (EMEA).

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