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NHS North West London Pathology

# Background Interfering Peaks in a Quantitative LC-MS/MS Dihydrotesterone Assay

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## Background Information

Dihydrotestosterone (DHT) is principally synthesised *in vivo* via the 5α reduction of testosterone. It is a potent activator of the androgen receptor and plays a major role in male sexual development.

The clinical indications for DHT measurement are primarily the diagnosis of 5α reductase deficiency in infants with disordered sexual development (DSD) and monitoring of patients taking oral testosterone supplements, which are metabolised to DHT in the gut before being absorbed.

## Method Information

- 150 µl of patient serum and dihydrotesterone-$^{13}C_3$ ($^{13}C_3$–DHT) internal standard (IS) solution mixed and extracted on a 96-well Oasis MAX µElution plate using a TECAN Freedom EVO-2 100.
- Waters ACQUITY UPLC system
- Waters TQ-XS mass spectrometer
- Mobile Phase A: 0.05 mmol/L Ammonium Fluoride in $H_2O$
- Mobile Phase B: 100% MeOH
- Cortecs® UPLCS® C18 1.6 µm 2.1 x 100 mm column with guard column
- UPLC gradient 60%A/40%B to 30%A/70%B over 3.5 min at a flow rate of 0.25 ml/min & column temperature 50°C.
- Injection volume: 15 µl
- Selective reaction monitoring (SRM) at 291.5>255.5 (DHT) and 294.5>258.5 ($^{13}C_3$-DHT).

## Problem

High background and interfering peaks were observed in the analytical retention time (RT) window for $^{13}C_3$-DHT transition (294.5 > 258.5) in some patient samples. No ion suppression was observed however the high background prevented the accurate integration of the $^{13}C_3$-DHT IS and subsequent DHT quantification (*Fig. 1*).

This interference was not observed during the method validation and affected a small, but increasing, number of patient samples.

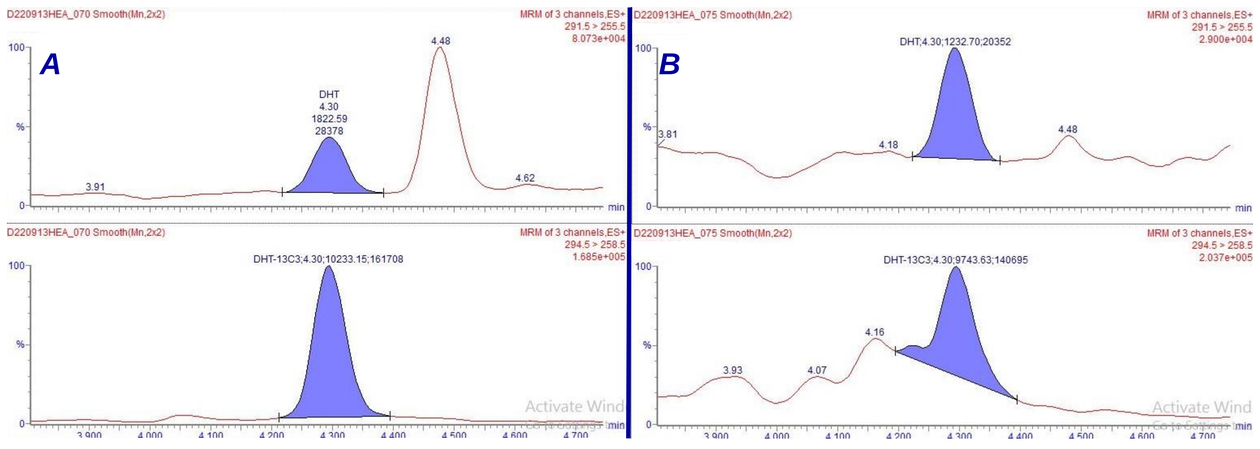


***Figure 1. Example chromatograms for an unaffected (A) and an affected (B) sample. Top panel shows transition 291.5>255.5 (DHT) and bottom panel shows transition 294.5>258.5 ($^{13}C_3$ –DHT).***

## Troubleshooting

Considered post-analytical processing

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Considered common sources of background/analytical interference:
- Mobile phase
- Glassware
- Lines
- Autosampler
- Guard column
- Column
- MS/MS tuning

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Considered pre-analytical factors:
- Patient factors (drugs, diet)
- Sample tube additives
- Sample handling (pre-extraction)
- Sample matrix
- Sample extraction
- Operator factors (hand creams, PPE)

Data acquisition and processing method is common for all samples therefore excluded.

Mobile phase freshly prepared into clean glassware & guard column changed. Repeat analysis of affected and unaffected samples confirmed that interference was reproducible and limited to specific samples i.e. analytical source of interference excluded.

Sample matrix, extraction & operator factors excluded as interference limited to specific samples.
Audit of 14 affected patient samples identified no common patient factors.
Experimental work performed to investigate sample tube and sample handling (pre-extraction) – see *Figure 2*.

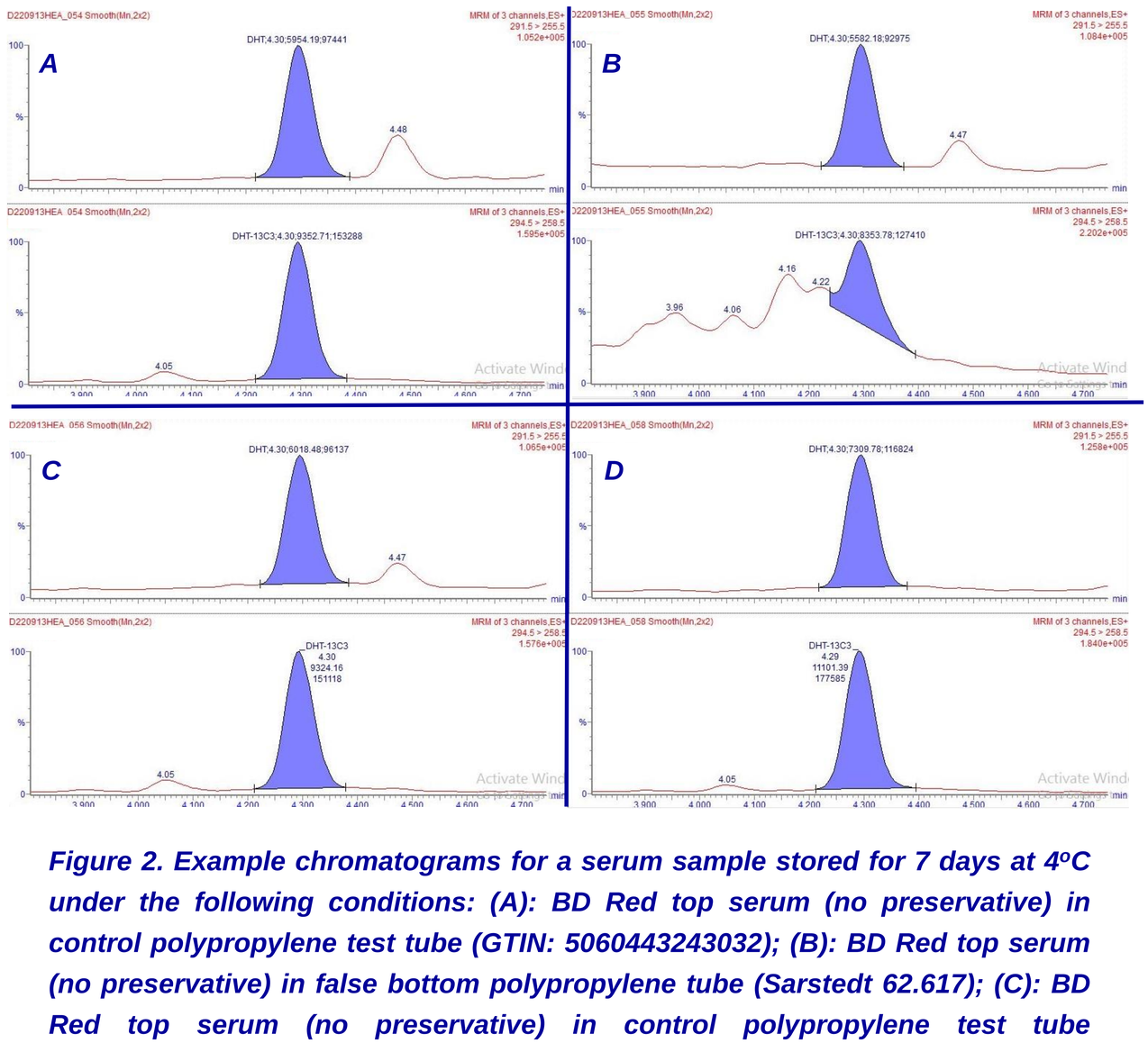


***Figure 2. Example chromatograms for a serum sample stored for 7 days at 4°C under the following conditions: (A): BD Red top serum (no preservative) in control polypropylene test tube (GTIN: 5060443243032); (B): BD Red top serum (no preservative) in false bottom polypropylene tube (Sarstedt 62.617); (C): BD Red top serum (no preservative) in control polypropylene test tube (GTIN: 5060443243032) with polypropylene pipette tip (Inpeco 0A00026280); (D): BD SST™ serum (gel preservative) in control polypropylene test tube (GTIN: 5060443243032). Top panel shows transition 291.5>255.5 (DHT) and bottom panel shows transition 294.5>258.5 ($^{13}C_3$ –DHT).***

## Outcome

Experimental work demonstrated that false bottom polypropylene tubes (Sarstedt 62.617) used on-board an Abbott a3600 Aliquoter Module were the source of the interference. Sample workflow was altered to avoid automated aliquoting into the affected tubes. The interference was not encountered again. This experience highlights the sensitivity of LC-MS/MS assays to sample workflow and consumable suppliers.

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