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Written by: Reviewed by:	*WADA* Science *WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
Date:	21 December 2020	Effective Date:	1 January 2021

DETECTION OF TULOBUTEROL IN THE PRESENCE OF BUPROPION

1.0 Introduction

WADA wishes to draw the attention of the Laboratories to the following observations and instructions on the analysis and reporting of **Tulobuterol** (1-(2-Chlorophenyl)-2-[(2-methyl-2-propanyl) amino] ethanol) (**Figure 1a**).

Tulobuterol is listed by name under section *S3*. beta-2 agonists of the *Prohibited List* and is available as an active ingredient of asthma medications in several countries.

Tulobuterol shares structural similarity with **Bupropion** (Figure 1), which is not a *Prohibited Substance*, but is included in the *WADA* Monitoring Program.

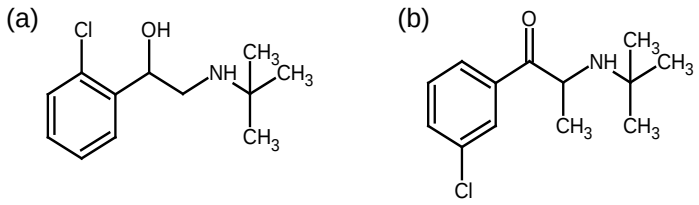

Figure 1. Tulobuterol (a) and bupropion (b) chemical structures.

Depending on the chemical environment, the bupropion structure may undergo various chemical modifications, which can lead to the formation of several degradation products (Figure 2).

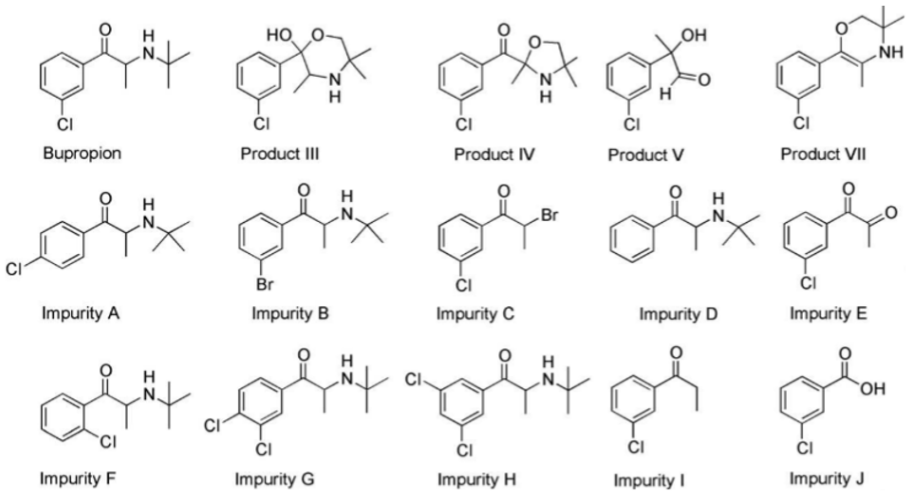

Figure 2. Structures of bupropion, its degradation-related impurities (III-V, VII) and US Pharmacopeia synthesis-related impurities (A-J).

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It has been determined that a signal derived from a bupropion derivative (either a degradation- or synthesis-related impurity, or a *Metabolite*) may interfere with the detection of tulobuterol (Figure 3).

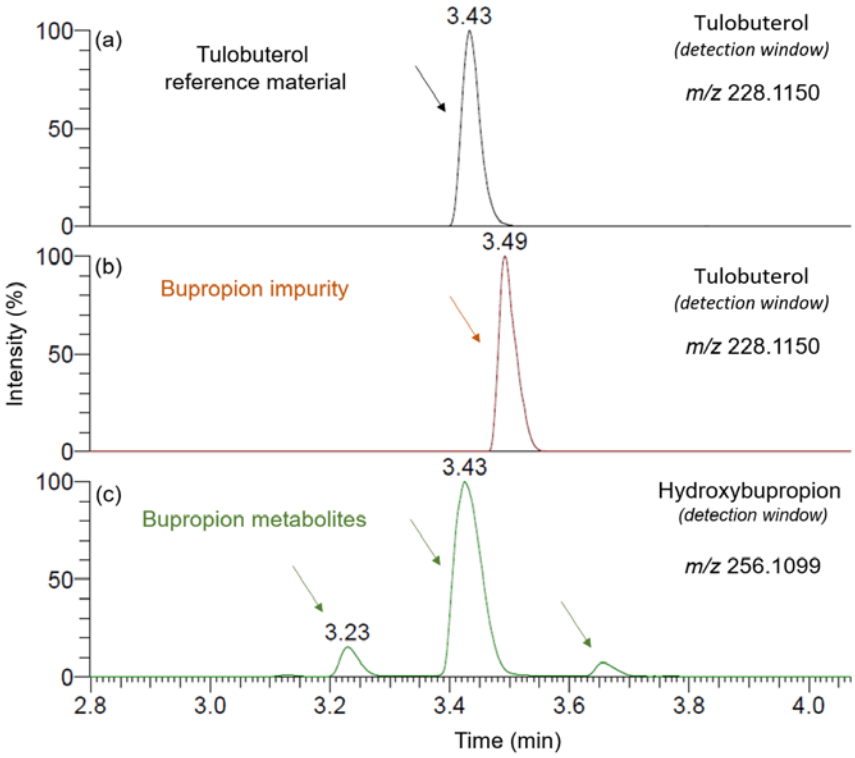

Figure 3. Liquid Chromatography — High Resolution Mass Spectrometry (LC-HRMS) analysis: a) Tulobuterol Reference Material (RM) spiked in blank urine, b) *Sample* containing the bupropion impurity, and c) Various hydroxylated bupropion *Metabolites*.

In the example above, the chromatographic condition allows the tulobuterol peak (3.43 min) to be resolved from the putative bupropion impurity (3.49 min). However, depending on the chromatographic setup, the bupropion impurity may coelute with tulobuterol. The structure of the bupropion impurity is currently unknown, but it has been suggested as a chloro-isomer of tulobuterol, although the fate of the α-methyl group from bupropion is currently unclear.

On LC-ESI-MS/HRMS, applying low collision energy (CE) (< 40 eV), the ion abundance ratios for tulobuterol and the coeluting bupropion impurity are nearly identical (Figure 4). Even a Confirmation Procedure (CP) including three (3) ion transitions may lead to comparable signal ratios.

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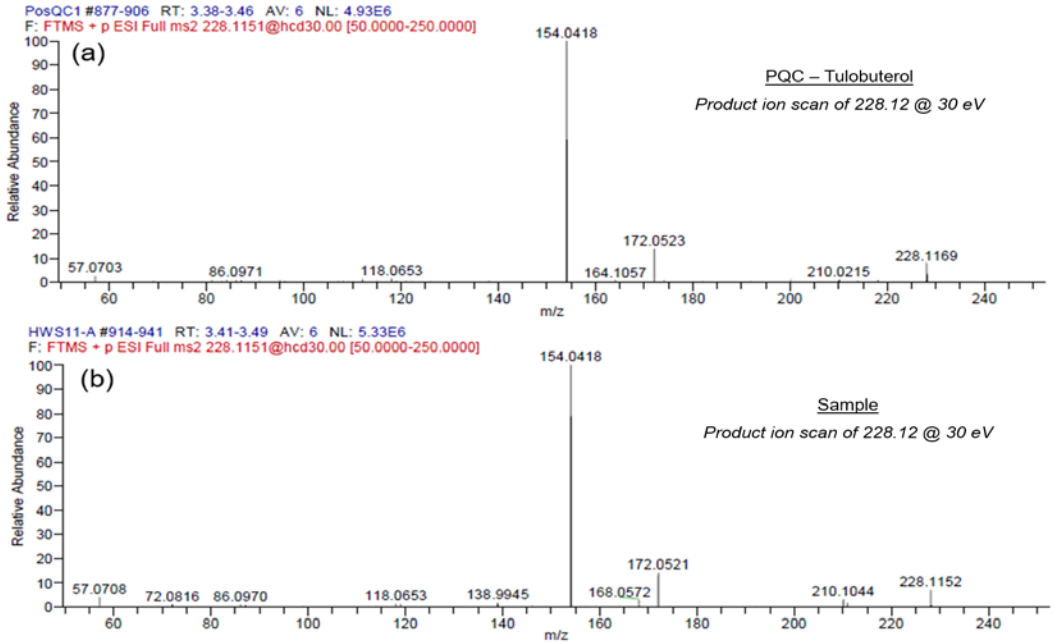

Figure 4. Mass spectrum comparison from product ion scan of *m/z* 228 at 30 eV: a) Tulobuterol-spiked positive quality control sample and b) *Sample* with a bupropion concentration higher than (>) 400 ng/mL.

Nevertheless, the differentiation between these two substances may be possible if a higher CE is applied during LC-ESI-MS/HRMS analysis (Figure 5). On the other hand, depending on the instrument setup, the product ion scan of *m/z* 228 at ≥ 40 eV may not result in differences in the ion intensities of *m/z* 118 and *m/z* 119.

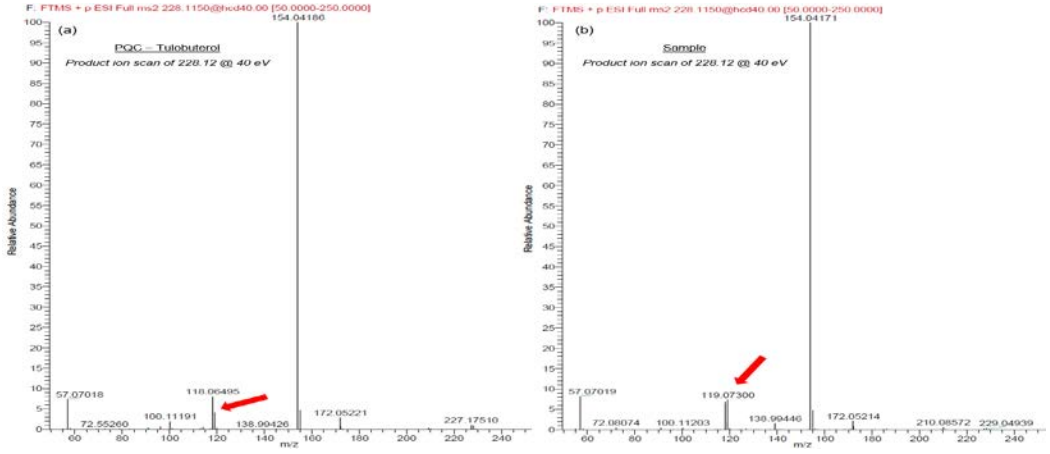

Figure 5. Mass spectrum comparison from product ion scan of *m/z* 228 at 40 eV: a) Positive quality control sample spiked with tulobuterol and b) *Sample* containing bupropion impurity.

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In addition, the presence of bupropion and/or its *Metabolites* at a very high concentration in the *Sample* may saturate the stationary phase and cause a shift of the expected retention times (RT) of tulobuterol and the interfering bupropion impurity. This phenomenon would not be observed in the tulobuterol positive quality control sample.

Thus, even if the Analytical *Testing* Procedure provides the necessary chromatographic resolution required for identification of tulobuterol in the presence of the bupropion impurity (*e.g.,* Figure 6c), the TD IDCR [1] RT criterion for tulobuterol, as defined in the positive quality control sample (Figure 6a), may be putatively met by the bupropion impurity present in the *Sample*, which elutes at the expected RT of tulobuterol (*e.g.,* Figure 6b).

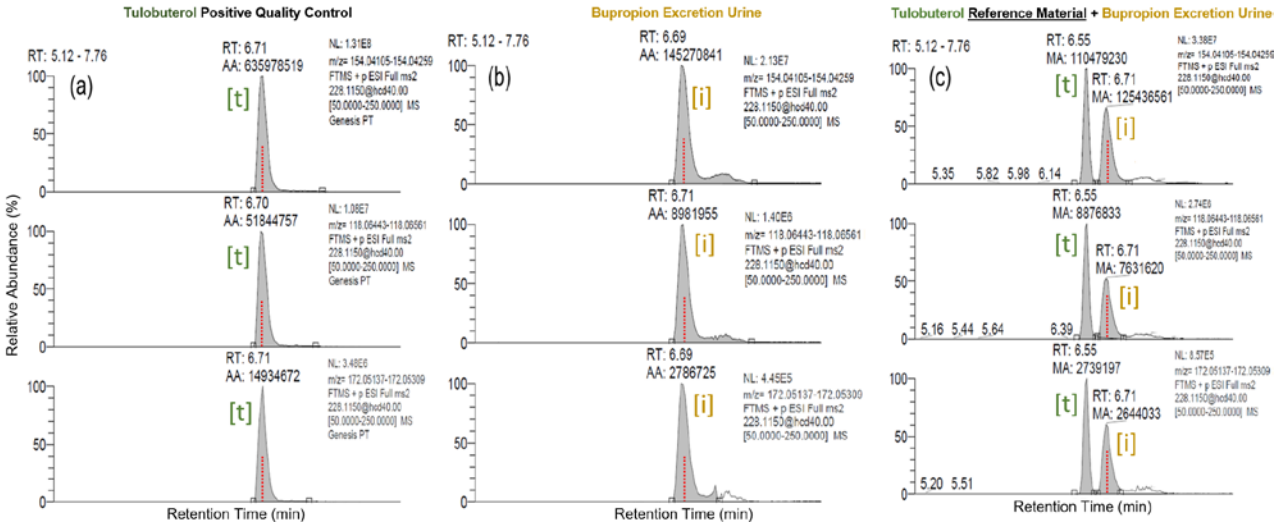

Figure 6. CP by LC-HRMS monitoring three (3) product ions of *m/z* 228.1 at 40 eV (*m/z* 154.04182, *m/z* 118.06502 and *m/z* 172.05223) and expected tulobuterol RT indicated by the red dashed line. a) Positive Quality Control (PQC; tulobuterol [t] at 5 ng/mL, RT = 6.71 min), b) *Sample* containing bupropion at a high concentration and bupropion impurity [i] at RT = 6.69 min, and c) the same *Sample* (b), spiked with tulobuterol standard and analyzed under the same conditions, in which the tulobuterol RT has shifted to 6.55 min, while the bupropion impurity elutes at 6.71 min. Thus, the bupropion impurity RT in this *Sample* is the same as that observed for tulobuterol in the PQC.

The chromatographic evaluation of the *Sample* after spiking it with the tulobuterol RM may assist in determining the "correct" RT of tulobuterol in the *Sample* (Figure 6c). Since the RT is dependent on the matrix, a comparison between an Aliquot which has been spiked with the tulobuterol RM and a non-spiked Aliquot would allow the Laboratory to detect either an emerging new peak for tulobuterol or an increase in the relevant tulobuterol peak area of the spiked Aliquot. This would indicate either the presence of the bupropion impurity or the "correct" RT of tulobuterol in the *Sample*, respectively.

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As for LC analysis, depending on the chromatographic setup, the analysis of tulobuterol by Gas Chromatography may risk a false *Adverse Analytical Finding* (*AAF*) if a bupropion interference is present at the expected tulobuterol RT (Figure 7).

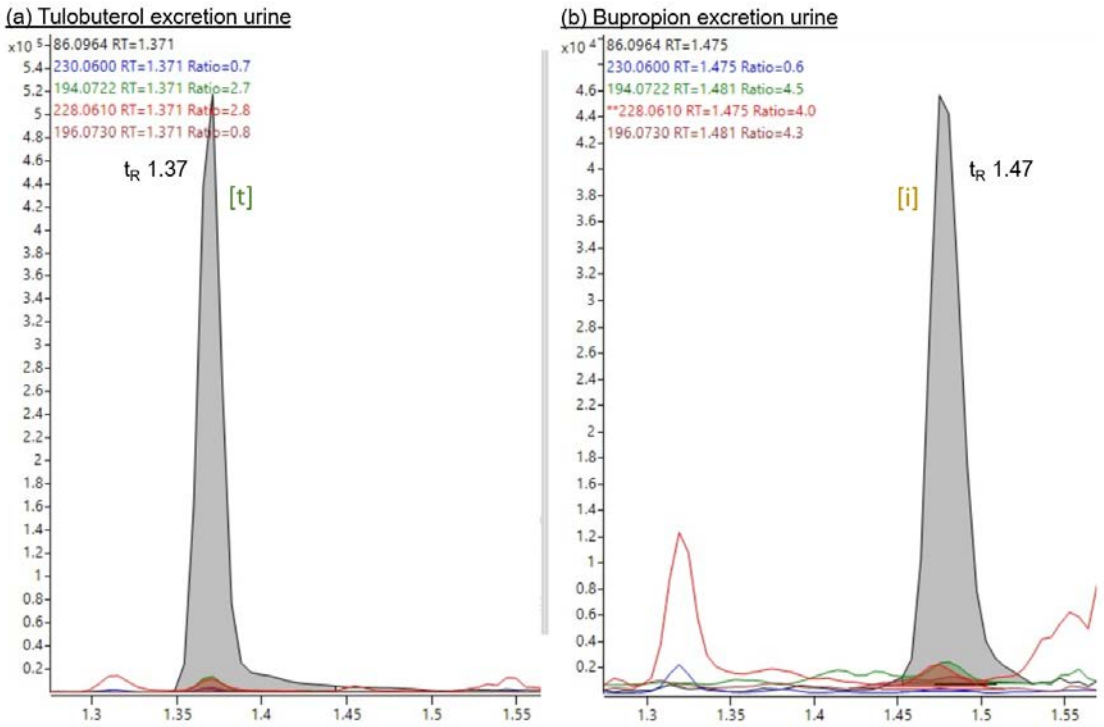

Figure 7. Gas Chromatography — High Resolution Mass Spectrometry (GC-HRMS) analysis: observation of bupropion interference in the tulobuterol monitoring window (*m/z* 86.0964, *m/z* 230.0600, *m/z* 194.0722, *m/z* 228.0610 and *m/z* 196.0730): a) Tulobuterol [t] excretion urine, and b) bupropion excretion urine presenting the impurity [i].

By GC-MS, the major tulobuterol ion is *m/z* 86.0964, followed by the *m/z* 230.0600, 194.0722, 228.0610 and 196.0730, all at less than 5% of the intensity of the major ion. In the present chromatographic conditions, tulobuterol elutes at a RT of 1.37 min (Figure 7a), while an excretion urine from a bupropion administration shows a peak eluting at 1.47 min, which has the same major ion transition of tulobuterol — identified as the bupropion impurity (Figure 7b). Because of the similarities in mass spectra and the small difference in RT between tulobuterol and the bupropion impurity, this suggests that there might be a risk of misidentification when analyzing tulobuterol by GC-MS if the chromatographic conditions do not enable a differentiation (peak resolution) by more than 0.1 min.

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2.0 Analysis and Reporting Requirements

Before reporting a result as an *AAF* for tulobuterol, Laboratories shall evaluate whether the finding is the result of a bupropion impurity interference.

1. The chromatographic and mass spectrometric setup shall consider the potential bupropion impurity interference. An excretion urine from a bupropion administration study should have been investigated by the Laboratory during the method validation of the tulobuterol CP. The analysis of a bupropion product or RM may not be sufficient for the detection of the interfering bupropion derivative. If mass spectral differentiation is not possible or ambiguous, the difference in RT between tulobuterol and any bupropion-derived interfering peaks should be greater than 0.1 min;

   [Comment: Preliminary studies have shown that the interfering bupropion derivative is only detected following an in vivo administration of bupropion, and not through the direct analysis of a bupropion-containing pill or RM.]

2. The correct RT of tulobuterol in the *Sample* shall be confirmed by the analysis of an additional Aliquot which has been spiked with tulobuterol RM. If an emergent new peak is observed in the tulobuterol-spiked Aliquot, the *Sample* shall be reported as a Negative Finding.

3.0 References

[1] *WADA Technical Document* TD IDCR: Minimum Criteria for Chromatographic-Mass Spectrometric Confirmation of the Identity of Analytes for *Doping Control* Purposes.

[Current versions of WADA Technical Documents may be found at https://www.wada-ama.org/en/what-we-do/science-medical/laboratories]