Qualitative doping analysis of β-blockers in urine by GC-MS/MS

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Introduction

The World Anti-Doping Agency (WADA) was established in 1999 as an international independent agency composed and funded equally by the sport movement and governments of the world.

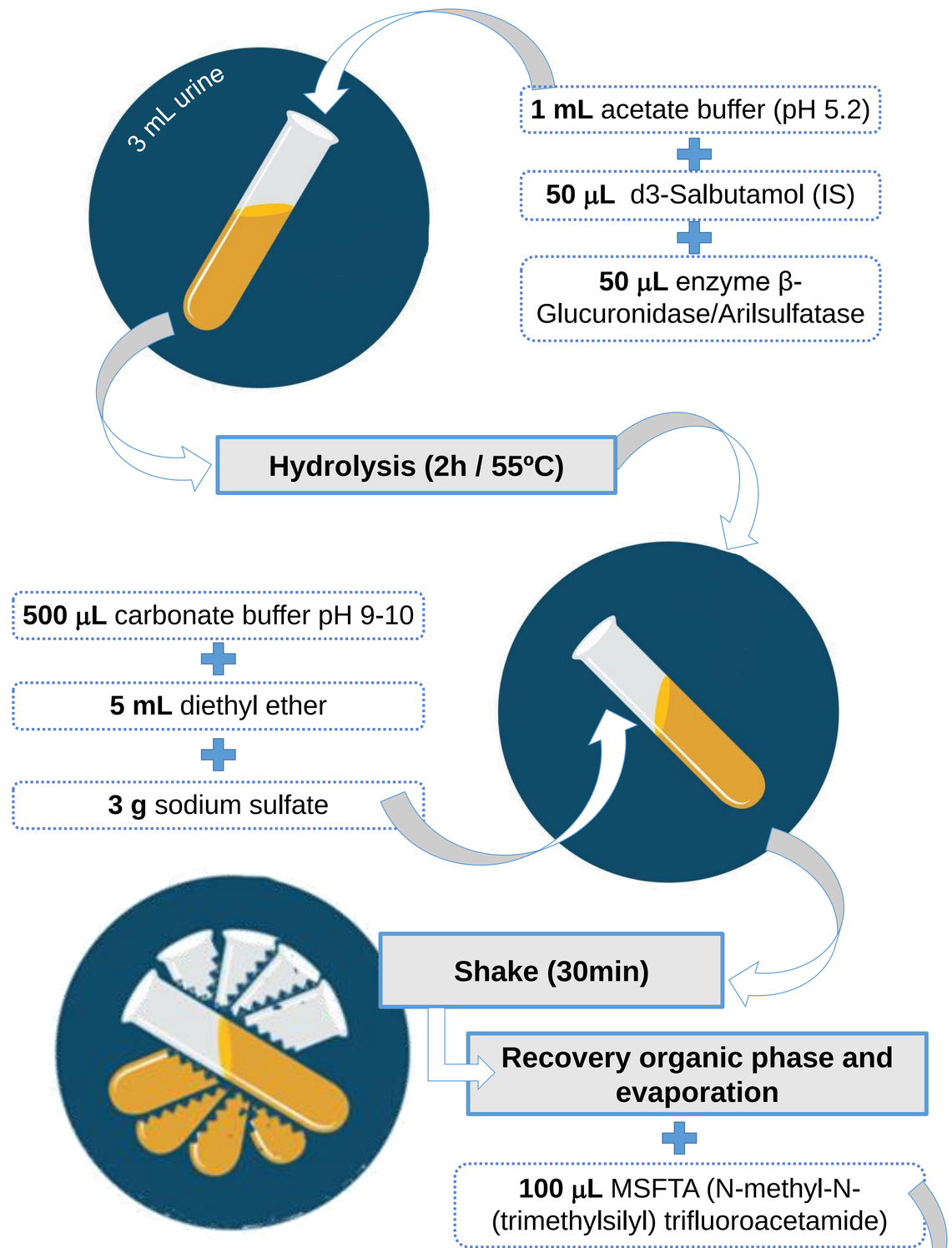
WADA stands by the slogan "Play True", and embraces it every year, becoming stricter and increasing the number of compounds included in the List of Prohibited Substances (annually reviewed). This extensive list describes the substances for witch the intake is forbidden to athletes that want to compete on events organized by Code Compliance Signatories.

WADA-accredited laboratories must have the ability to detect the over 400 enhancing drugs in different biological matrices (urine and blood). The strategy is to screen for these substances in faster comprehensive methods, usually by LC-MS/MS and GC-MS/MS, to clearly distinguish negative samples form suspected ones and then to use more specific/selective methods to confirm the presence/absence of any prohibited drug.
One of the classes that are prohibited in-competition and out-of-competition in some sports (e.g., automobile, golf, archery, shooting, etc.), are β-blockers.

β-blockers - beta-adrenergic receptor blockers - are a class of drugs widely used in clinical pharmacology to treat cardiovascular diseases and related conditions (e.g. controlling acute panic symptoms in anxiety-provoking situations). These drugs reduce blood pressure, manage cardiac arrhythmias and are cardioprotective after myocardial infarction (heart attack). β-blockers are competitive antagonists that bind to beta-adrenoceptors and block the receptor sites for the epinephrine (adrenaline) and norepinephrine (noradrenaline) of the sympathetic nervous system [1-5].

In the illicit pharmacological support to sport competition, β-blockers are used to reduce the cardiac frequency and to minimize tremors, in order to improve the performance in skill-based sport disciplines (ex. automobile, golf, shooting).

Methodology



Agilent 7890B Triple Quadropole 7000D GC/MS System.
Column: Agilent, HP-Ultra 1, length 25 m, i.d. 0.2 mm, film thickness 0.11 µm. Carrier gas: helium, 25.95 psi. Injector: 280 ºC, split 10:1, injection volume 3 µL. Temp. Program: 180 ºC (0 min), 4 ºC/min to 240 ºC (3 min), 40 ºC/min to 320 ºC (2 min).

Results and Discussion

Figure 1 shows the chromatograms for the nine (9) β-blockers included in this validation method: metoprolol, propranolol (and its metabolite 4-hydroxy propranolol), timolol, sotalol, celiprolol, bisoprolol, atenolol and nebivolol at the minimum required performance level (MRPL) of 100 ng/mL. [6]

The limit of detection (LOD) for all analytes was determined as 50 ng/mL. According to WADA's technical document TD2019MRPL, β-blockers should not be reported as an Adverse Analytical Finding (AAF) at levels below than 50 ng/mL.[7]

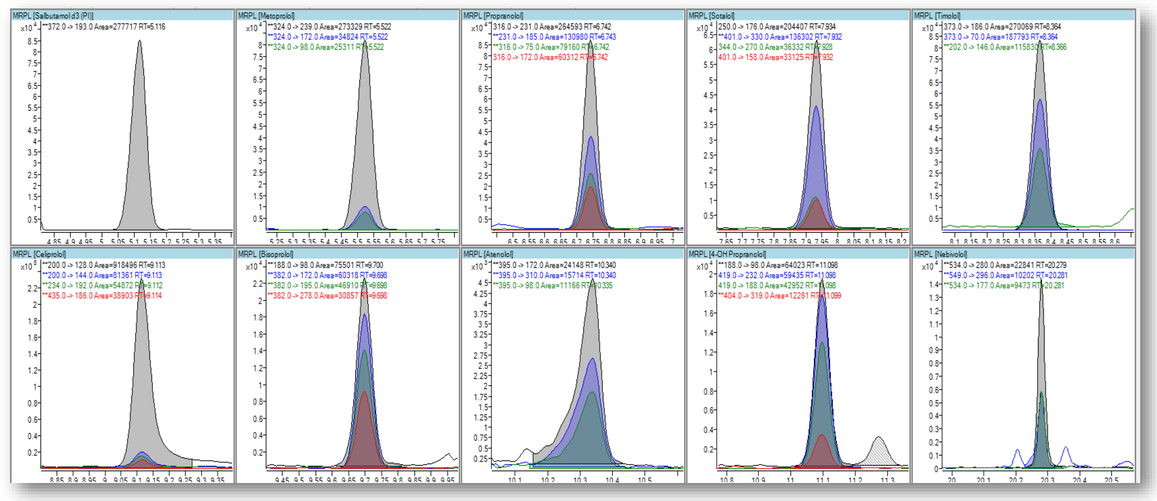


Figure 1 – Chromatograms of β-blockers included in the qualitative method

Nineteen (19) blank samples were fortified at MRPL and analysed in order to evaluate the selectivity and identification capacity of the method.
The method shows a good selectivity (0 % of false positives). Regarding to the identification capacity 5% of false negative for celiprolol, sotalol, atenolol, 4-hydroxy propranolol, bisoprolol and 10 % for timolol were identified.

Table 1 – Recoveries for all analytes at two levels: MRPL and 4x MRPL.

Compound	Low level (%)	High level (%)
Metoprolol	92	86
Propranolol	90	87
Sotalol	50	42
Timolol	87	84
Celiprolol	73	141
Bisoprolol	90	88
Atenolol	45	46
4-OH Propranolol	24	18
Nebivolol	99	82

The validation method shows good recoveries for the two levels tested. For the low level recoveries the method shows a range between 45-99 % and for the high level between 42-141 % (Table 1).
However, 4-hydroxy-propranolol shows recoveries of 18 % and 24 % for low and high level, respectively. In this case, these values are not relevant once the determined LOD for this analyte is in accordance to the TD2019MRPL specifications and at both levels. No carryover has been verified.

Two positive samples for β-blockers from interlaboratory tests were analysed by the validated method and the results were according to the expectable and meeting all the identification criteria.
To these extracted samples, were applied a stability test: the sequence were injected after 42h and after 83h and the results were in accordance to the first sequence and to the WADA's reporting rules.

Conclusion

The method proved to be selective, robust and presents good identification capacity where the LOD determined for all substances was 50 ng/mL. No carryover has been verified and the method presents adequate recoveries of the analytes.

Once completed the validation, the method was subsequently applied to two interlaboratory positive samples for β-blockers and the results were in accordance with WADA's reporting rules, so the developed method can be an important new tool to confirm the presence of the β-blockers analyzed and being under flexible scope of accreditation (in compliance with NP EN ISO/IEC 17025) it can be easily incremented with other β-blockers.

References:

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