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Analysis of tetrahydroisoquinoline β_2-agonists by GC-MS-ITD

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Introduction

The use of β_2-agonists by athletes is restricted by the International Olympic Committee (IOC) due to their anabolic and stimulating effects[1]. Several methods are decribed in the literature for confirmation of β_2-agonists in human urine. The evaluation of the biotransformation process indicates that, with the presence of activating substituents (such as hydroxy groups) in positions 3 and/or 5 of their phenyl moiety, β_2-agonists are excreted as tetrahydroisoquinoline (THIQ) derivatives, that are described as phase I metabolites[2]. So, orciprenaline, isoprenaline and terbutaline may be excreted as unchanged, sulfoconjugated and as THIQ derivatives. Usually the Pictet-Spengler reaction (Figure 1) is used as a synthetic way to obtain THIQ derivatives[2-4]. Gas chromatography/ion trap detector (GC-ITD) has shown high sensitivity and selectivity for several doping agents, becoming an interesting option for confirmation of exogenous substances[5].

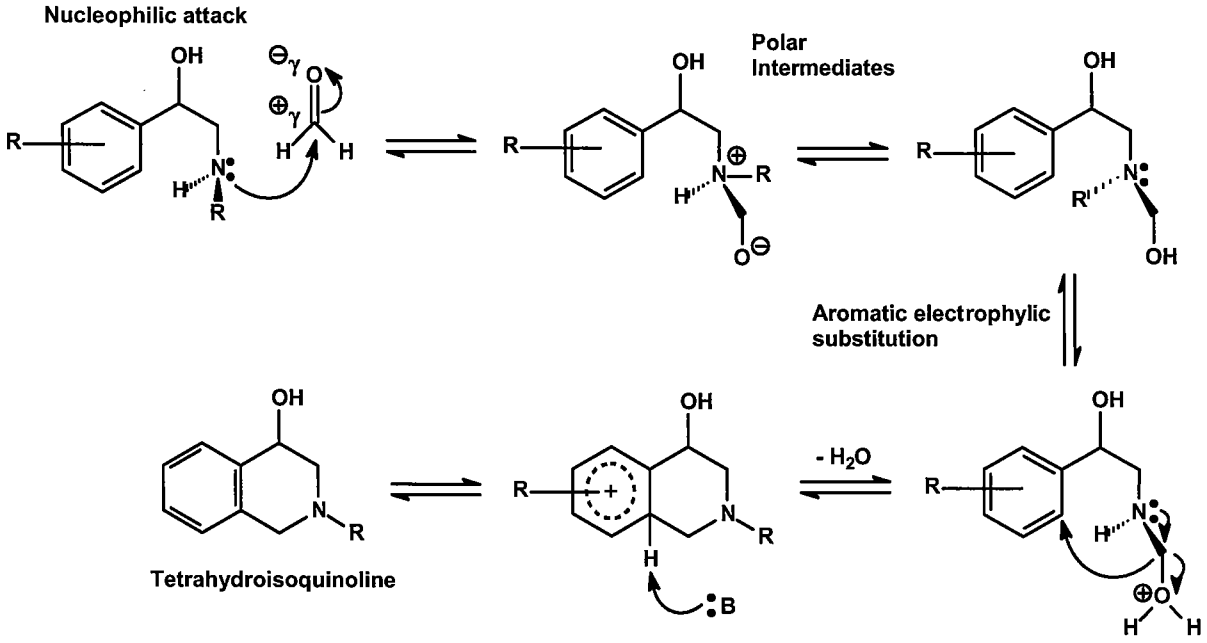

Figure 1: Scheme for formation of THIQ derivatives through Pictet-Spengler reaction.

Our goal is to evaluate the use of GC-ITD in confirmation analysis of orciprenaline, isoprenaline and terbutaline as THIQ-TMS after derivatization with formaldehyde, followed by silylation with MSTFA:NH_4I:2-mercaptoethanol.

Experimental

Sample Preparation: Urine samples were processed according to the procedure described by Henze et al[2]., for screening analysis of β_2-agonists.

Instrumental conditions: The main instrumental GC-MS-ITD conditions are summarized in table 1.

Table 1. GC/MS-ITD iInstrumental conditions.

Gas Chromatograph (Varian 3800)	
Column:	Crosslinked Methyl Silicone Gum
	17m x 0.2mm x 0.11μm film
Flow Rate:	1mL/min / Pulse pressure 25 psi (0.85 min)
Injection Temp*.	280°C
Transfer Line Temp.	280°C
Injection Mode	Split, 1:20
Injection volume	3μL
Oven Program :	140°C, 20°C/min until 320°C
(Total time = 12 min)	
Spectrometric parameters (Varian 2000)	
Target Tic	20000 counts
Max Ionization Time	25000 ms
Background Mass	90 m/z
Ion Trap Temp.	220°C
Amplitude	55 (volts)
Wave form	Non-resonant
RF (m/z)	81
Fragmentation	Collision-induce dissociation (CID)

*Temp = Temperature.

Results

Due to their similar structures and retention times (t_R) orciprenaline, isoprenaline and terbutaline derivatives were optimized with the same CID parameters. The chromatographic conditions used for the analysis of THIQ-TMS derivatives resulted in time saving, good resolution and very symmetric peaks. The CID parameters allows the detection of orciprenaline and terbutaline in 1ppb with a signal / noise ratio higher than 3 (Figures 2 and Table 2).

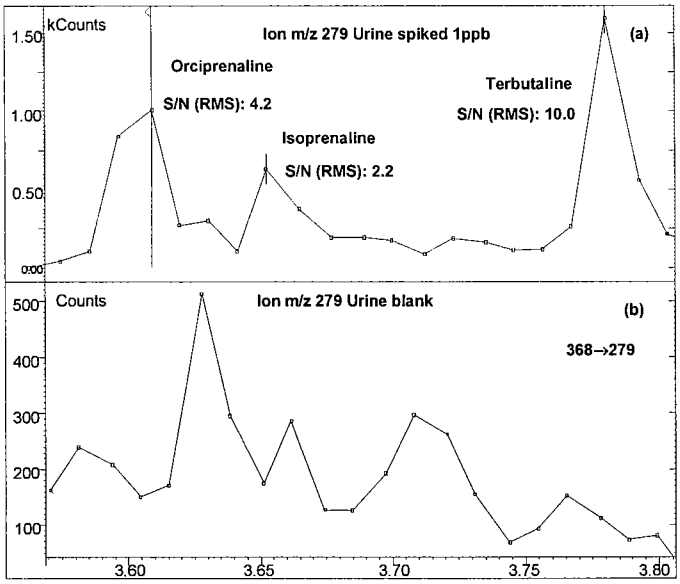

Figure 2. GC-MS-MS (ITD) fragment of m/z 279 from analysis of (a) urine sample spiked with 1 ppb of orciprenaline, isoprenaline and terbutaline and (b) blank urine. Amplitude 55 (volts), wave form non-ressonant, RF 81m/z, CID fragmentation.

Table 2. Parent ion, daugther ions, retention time, CID parameters for analytes and signal / noise ratio at 1ppb level in fortified urine.

Analytes	Parent Ion	Ion (intensity relative in %)	t_R (min)	S/N Ration
Orciprenaline	368	279 (100%), 265 (72%), 251(70%)	3.61	4.2
Isoprenaline	368	265 (100%), 279 (71%), 251 (35%)	3.65	2.2
Terbutaline	368	279 (100%), 251 (61%), 265 (53%)	3.78	10.0

Despite of the S/N ration in 1ppb level is below 3, the detection limit for isoprenaline should be near 2 ppb. The lower level of detection reached by the GC-MS-ITD is better than the method low-resolution quadrupole MS (10 ng/mL)[2]. As indicated for Henzel et al.[2] the

Henzel et al.[2] the ciclization step does not interfere with the analysis of the β_2-agonists which structure do not permit THIQ formation, such as clenbuterol, cimaterol, cimbuterol, etc.

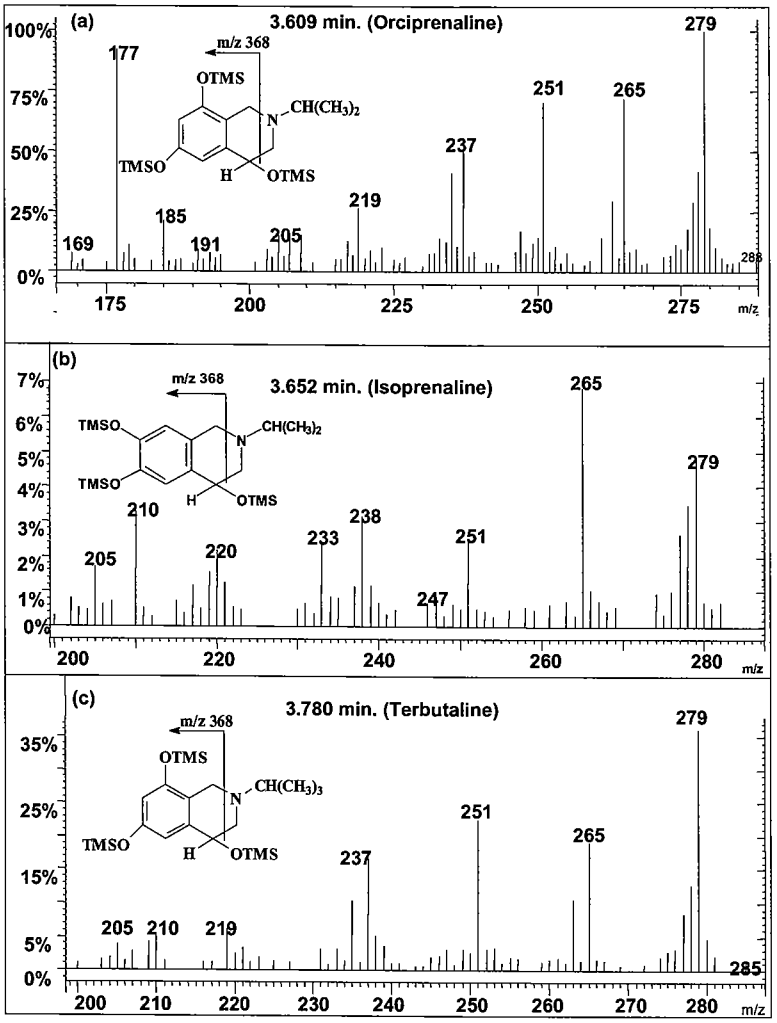

Figure 3. Full CID GC-MS-MS (ITD) spectra of (a) orciprenaline, (b) isoprenaline and (c) terbutaline THIQ-TMS. Amplitude 55 (volts), wave form non-ressonant, RF 81m/z, CID fragmentation.

The amount of formaldehyde necessary to convert all analytes in THIQ derivatives needs to be optimized. As formaldehyde is a final product of catabolism it is eliminated in urine in different amounts depending on diet, ingestion of drinks and ethnic groups. A preliminary study in urine from Brazilian athletes showed that the formation of β_2-agonists THIQ derivatives was of 100% at the concentration of formaldehyde $\geq$ 0.08mg/mL of urine (Figure 4). GC-MS Agilent was used for this evaluation. This value is superior to the one described by Henze et al. (0.02mg/mL).

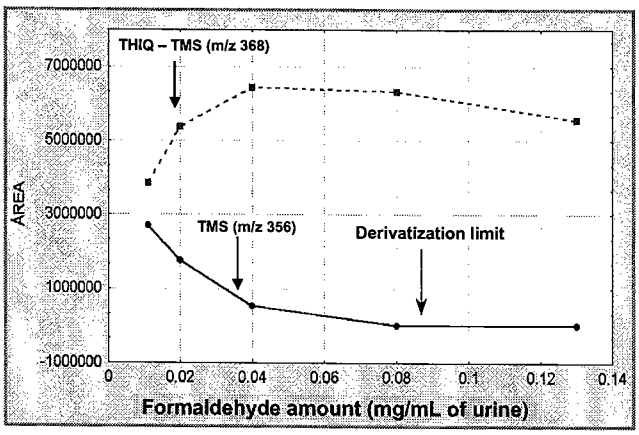

Figure 4. Percentage of THIQ derivatives x silyl derivatives as a function of formaldehyde concentration (0.01, 0.02, 0.04, 0.08, 0.13 mg/mL) in urine.

Conclusion

These results indicate the potentiality of the technique as an independent confirmation procedure for these analytes due to their peculiar structure, increased S/N and decrease of the detection limits. From the best of our knowledge, this is the first report on the identification and quantification of tetrahydroisoquinoline derivatives of the β_2-agonists performed by GC-ITD. This approach can be used for confirmation of other β_2-agonists with activating substituents in positions 3 and/or 5 of their phenyl moiety, as fenoterol and isoetarine. At a first glance, the appropriated amount of formaldehyde to allow the maximum yield of the ciclization step requires a special attention, including the dietary behaviour and ethnic source.

Reference

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