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Detection of deacetylmetipranolol arising from storage of standard solution of metipranolol

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Abstract

Metipranolol is a beta-blocker in the WADA list of substances prohibited in particular sports. It is converted to deacetylmetipranolol by the first pass metabolism and the parent compound is not excreted. A standard methanolic solution of metipranolol can be converted to deacetylmetipranolol during prolonged storage at -20°C. Spiking blank urine with this aged standard can lead to misinterpretation of the form of metipranolol in a suspected urine sample since the normal GC-MS screening procedure for derivatized beta-blockers cannot separate this metabolite from the parent compound. A specific GC-temperature program is required. However a LC-MS/MS method can separate metipranolol and its metabolite.

Introduction

Metipranolol is a beta-blocker in the WADA list of prohibited substances (Class P2, substances prohibited in particular sports) [1]. It is converted to deacetylmetipranolol by the first pass metabolism (Phase I) and the parent compound is not excreted [2,3]. However we have found that a stock solution of metipranolol in methanol can be converted to deacetylmetipranolol after storage for two years at -20°C. The normal GC-MS screening procedure for the O-TMS, N-TFA derivatives of beta-blockers cannot separate deacetylmetipranolol from metipropanolol. A specific temperature program for the GC was developed to separate the two compounds. This was confirmed using a LC-MS/MS method.

Experimental

Methanolic solution of metipranolol is unstable when stored for two years at -20°C. It is converted to deacetylmetipranolol. GC-MS separation of the TMS and TFA derivatives of metipranolol and deacetylmetipranolol can only be achieved by using a specific slow oven temperature program. However the two compounds are readily separated by LC-MS/MS. Since only deacetylmetipranolol is excreted metipranolol should not be added in control urine employed in screening procedure.

Results and Discussion

A stock solution of metipranolol in methanol, stored for two years at -20 ºC, was found to be unstable. Direct analysis of the stored stock solution, without the extraction step, was performed using both GC-MS and LC-MS/MS methods. The O-TMS, N-TFA derivatives of metipranolol and deacetylmetipranolol can only be separated using GC-MS with the slow oven temperature program (Table 1).

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GC/MS method

Instruments	Instrument parameters
GC (Agilent 6890)	**GC Column** :100% Dimethylpolysiloxane , L20m x I.D. 0.25 mm, F.T 0.10 µm
	Oven temperature : Initial 120° C, raising 10 °C/min to 300° C hold 1 min
	Spit ratio : 10:1
	Inject volume : 2 µl
MS, Single Quadrupole (Agilent 5973)	**Full scan mode** Mass range : 70-550
Run time	19 min

LC/MS/MS method

Instruments	Instrument parameters
HPLC (Shimadzu LC20 Prominence)	**LC Column** : Luna 3µm C18, 100 x 2.0 mm
	Mobile phase : Isocratic:70% A (0.1% formic acid in 5 mM ammonium acetate), 30% B (acetonitrile), flow rate 0.3 ml/min
	Inject volume : 5 µl
	Column oven : 40°C
MS/MS , Triple Quadrupole (Shimadzu LCMS-8030)	**MS polarity** : positive **Q1 Scan mode** : Mass range 250-320 **Product ion scan mode** : Precursor ion 268→ mass range :100-270 (Deacetylmetipranolol) Precursor ion 310 → mass range :100-320 (Metipranolol)
Run time	5 min

Table 1: GC/MS and LC/MS/MS parameters for detection of metipranolol and deacetylmetipranolol.

The retention times are 10.897 min and 10.942 min, respectively (Figure 1a). The mass spectrum showed only a very small abundance for M^+(m/z 477) of metipranolol-O-TMS-N-TFA and M^+ (m/z 507) for deacetylmetipranolol-bis-O-TMS-N-TFA, respectively (Figure 1a). The results were confirmed with LC-MS/MS. The retention times of metipranolol and deacetylmetipranolol are 2.101 min and 1.091 min, respectively (Figure 1b). The product ion mass spectrum showed $[M+H]^+$ (m/z 310) for metipranolol and $[M+H]^+$ (m/z 268) for acetylmetipranolol (see Figure 1b).

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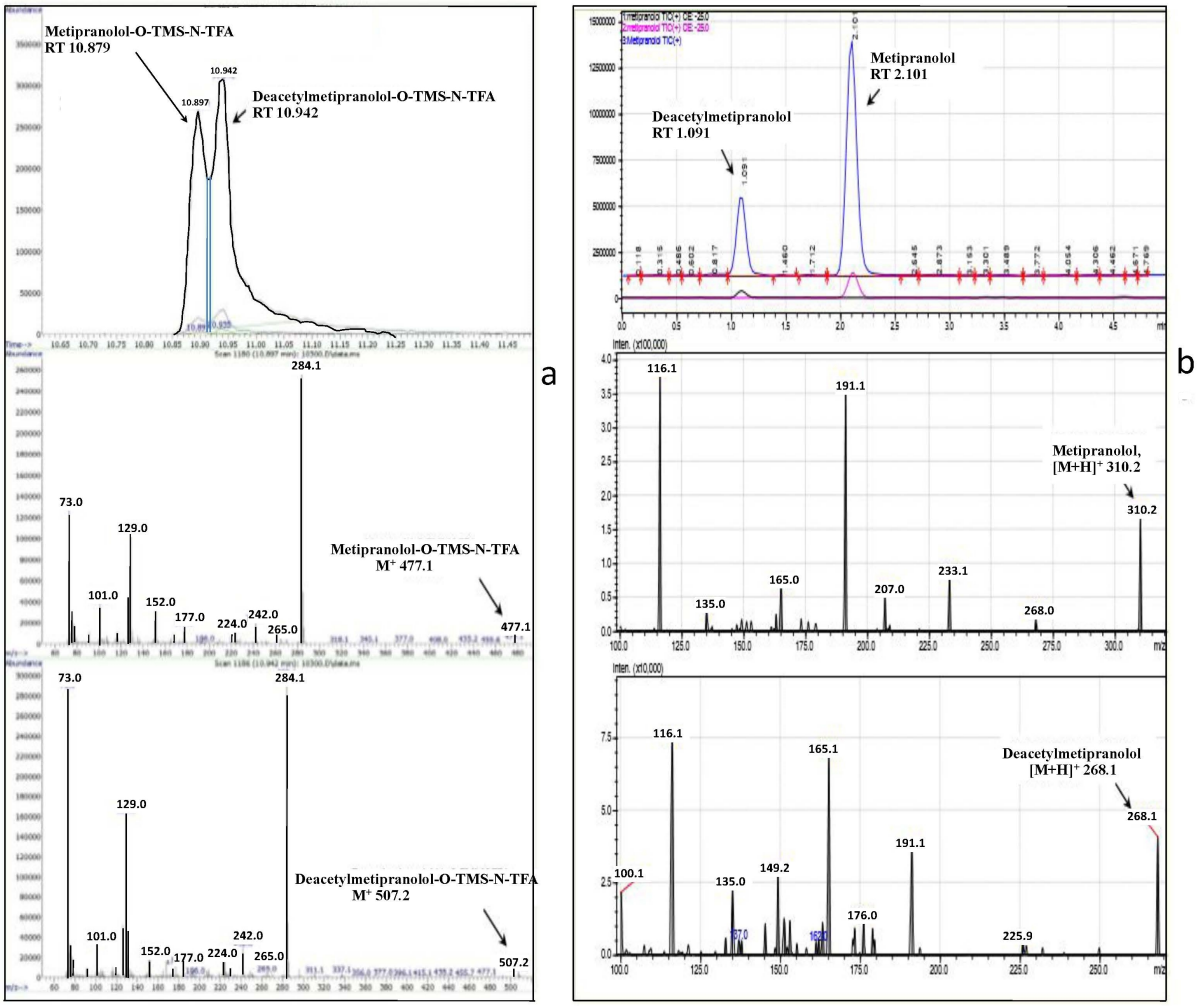

Figure 1: Chromatograms and mass spectra of pure standard metipranolol stored for two years. (a) Extract ion chromatogram and mass spectra of metipranolol-O-TMS-N-TFA and deacetylmetipranolol-bis-O-TMS-N-TFA by GC/MS. (b) Total ion chromatogram and product ion mass spectra of metipranolol and deacetylmetipranolol by LC-MS/MS.

The TMS and TFA derivatives of metipranolo and deacetymetipranolol are produced according to the reaction scheme shown in Figure 2. Thus both GC-MS and LC-MS/MS analyses showed conversion of metipranolol to deacetylmetipranolol on storage. Further a blank urine was spiked with a freshly prepared standard solution of metipranolol, extracted and analyzed by both GC-MS and LC-MS/MS. Only metipranolol was observed (see Figure 3). Using a normal GC-MS screening method for detection of betablockers in which metipranolol and its metabolite cannot be separated, the peak for acetylmetipranolol may be wrongly interpreted if the control urine was spiked with an aged standard solution of metipranolol. The abundance of the molecular ion is not easily observed (see Figure 1a). However the two compounds are well separated and identified by liquid chromatography (see Figure 1b). An analysis of an excretion urine showed only deacetylmetipranolol, confirming previous studies [2,3].

It should be noted that in many papers [4-6] on the screening of betablockers (and other prohibited substances) by LC-MS/MS and/or GC-MS include metipranolol in the list of betablockers extracted from urine without mentioning that only deacetylmetipropanolol is found in an excretion urine.

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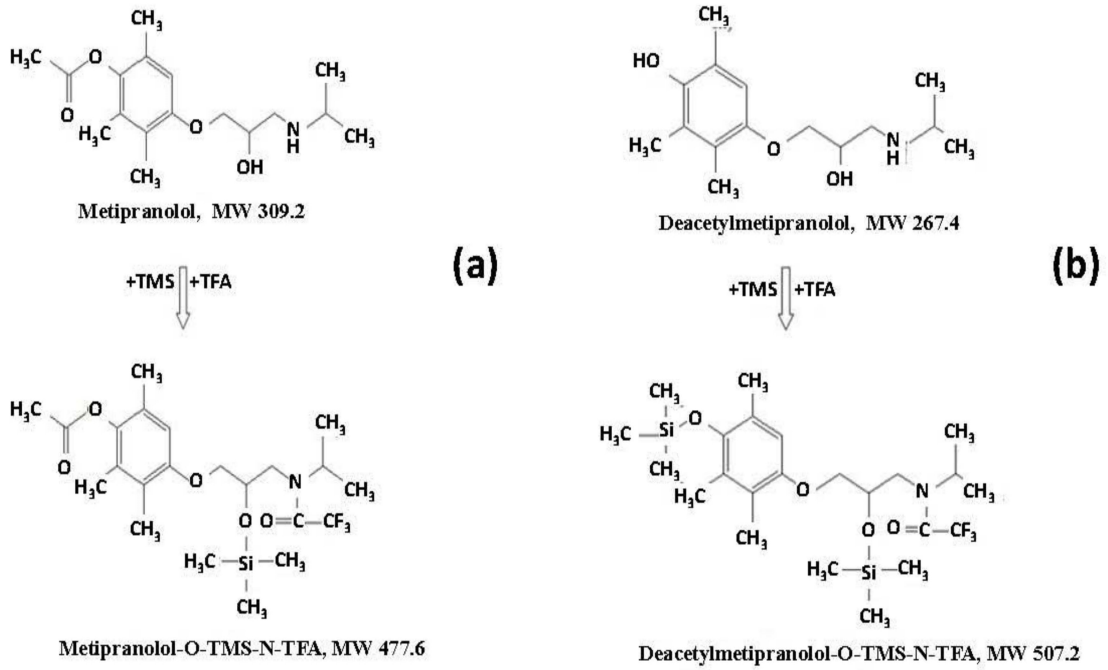

Figure 2: Chemical structures of (a) metipranolol and its derivative, metipranolol-O-TMS-N-TFA, (b) deacetylmetipranolol, and its derivative, deacetylmetipranolol-bis-O-TMS-N-TFA.

Conclusions

Methanolic solution of metipranolol is unstable when stored for two years at -20°C. GC-MS separation of the TMS and TFA derivatives of metipranolol and deacetylmetipranolol can only be achieved by using a specific slow oven temperature program. However the two compounds are readily separated by LC-MS/MS. Since only deacetylmetipranolol is excreted metipranolol should not be spiked in control urine.

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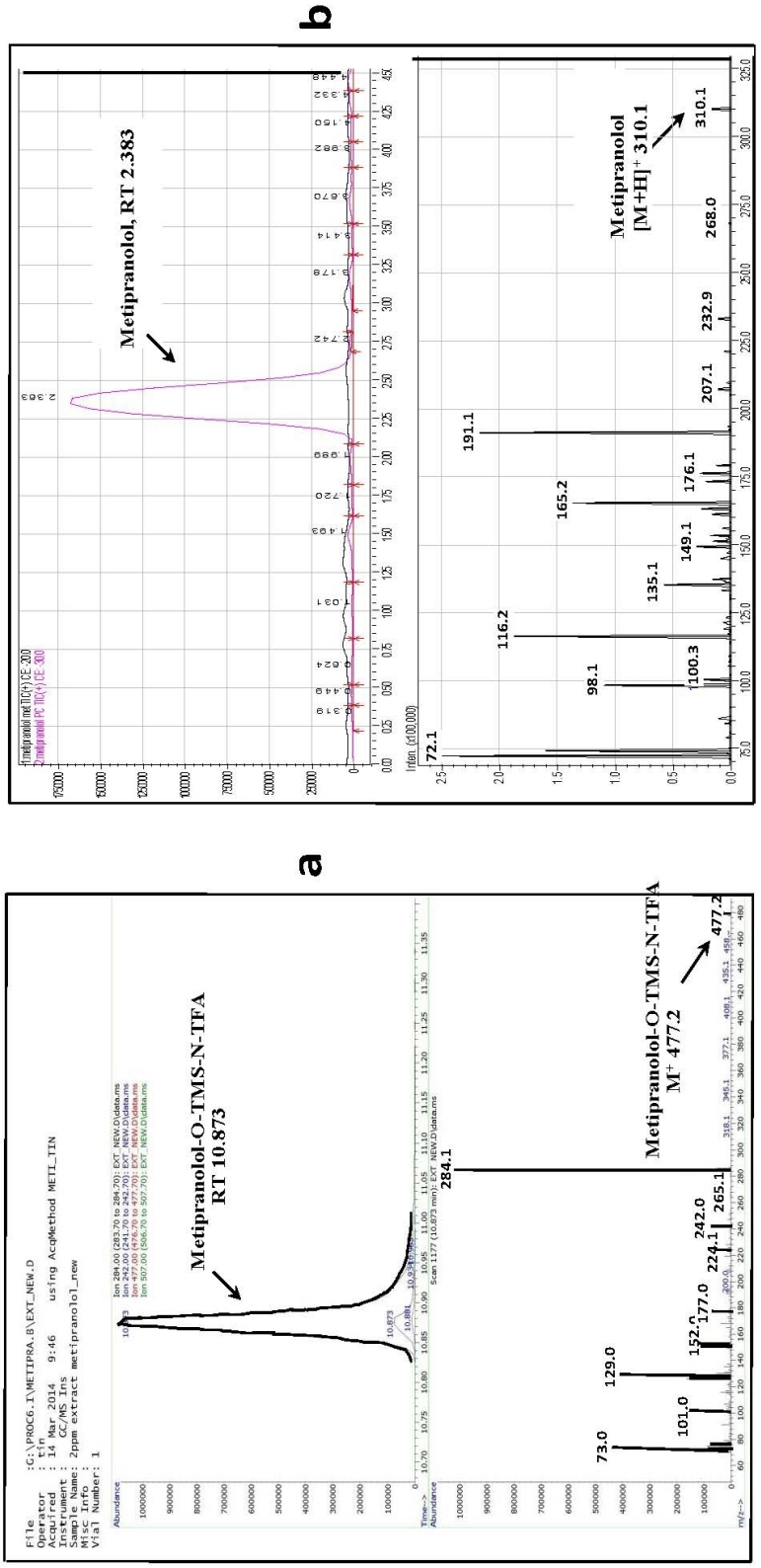

Figure 3: (a) Extract ion chromatogram and mass spectrum of metipranolol-O-TMS-N-TFA by GC/MS of blank urine spiked with freshly prepared standard metipranolol solution, (b) Total ion chromatogram and product ion mass spectrum of metipranolol by LC-MS/MS of blank urine spiked with freshly prepared standard metipranolol solution.

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