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## **In vitro and in vivo metabolism of RAD140, a novel non-steroidal SARM**

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### **Abstract**

The selective androgen receptor modulator RAD140 has been recently developed as a potent and orally bioavailable drug intended for the treatment of muscle wasting condition related to various malignant diseases. The experiments on an *in vitro* and *in vivo* metabolism of RAD140 (synthesized in-house) were conducted, which showed that this compound is metabolically stable. Using liquid chromatography – electrospray mass spectrometry in negative ionization mode, unchanged RAD140 can be detected in urine at least 8 days after administration of a single oral dose of 10 mg.

### **Introduction**

RAD140 (2-chloro-4-({(1*S*,2*R*)-1-[5-(4-cyanophenyl)-1,3,4-oxadiazol-2-yl]-2-hydroxypropyl}amino)-3-methylbenzonitrile) was recently introduced by Radius Health Inc. as a potent, orally bioavailable, non-steroidal selective androgen receptor modulator (SARM). This compound demonstrated promising results in the preclinical animal tests [1] and may subsequently enter human clinical trials. Its appearance on the black market cannot be excluded as well. Recently, Thevis et al. reported the mass spectral data for this SARM [2]. As the class of SARMs is prohibited by World Anti-Doping Agency, the antidoping laboratories are encouraged to include as many representatives of this class as possible.

### **Experimental**

*In vitro metabolism:* pooled mixed gender human liver microsomes (HLM) from BD Gentest (Woburn, MA, USA); incubations according to manufacturer's protocol.

*Instrument:* triple quadrupole mass spectrometer TSQ Vantage (ThermoFisher Scientific, San Jose, CA, USA) coupled to a liquid chromatograph Acquity (Waters, Milford, MA, USA).

*Separation:* Acquity BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) maintained at 60°C and protected by a Vanguard column (20 mm × 2.1 mm). The mobile phase flow rate 0.35 mL/min. Elution program: 0.5-min isocratic step at 95% of 0.1% formic acid in water (A) and 5% of 0.1% formic acid in methanol (B), linear increase to 95% of B within 4.5 min, hold at 95% of B for 2.5 min and then re-equilibration until the end of analysis (10 min).

*Detection:* heated electrospray ion source (HESI II); positive and negative ions. The collision gas pressure 1.5 mTorr (argon 99.9995%). The vaporizer and capillary temperature 370 and 300°C, respectively, spray voltage 4000 V.

### **Results and Discussion**

Non-steroidal SARM RAD140 (Fig. 1) was synthesized in-house according to [1] and first characterized by NMR spectrometry (data not shown), which was consistent with the structure. Liquid chromatography – triple quadrupole mass spectrometry was then applied to acquire respective ESI spectra. It was found however that in positive ESI on Thermo TSQ Vantage this compound produces very stable sodium adduct with only traces of protonated molecular ion, making impossible detection of RAD140 using positive ESI. Once switched to negative ESI mode, some in-source fragmentation was noticed, but it did not impede the detection of this compound.

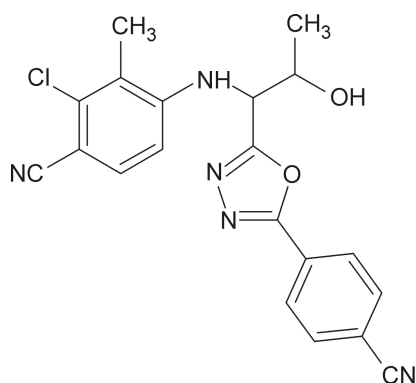


Fig. 1. Structure of RAD140

RAD140 was first subjected to an *in vitro* experiment to evaluate its metabolic profile. This compound was found to be very stable, with only low amounts of mono- and di-hydroxylation products formed. For these metabolites the same mass spectrometric behavior was observed: positive ESI was almost useless. Mass spectra of RAD140 and its monohydroxy metabolite are presented in Fig. 2.

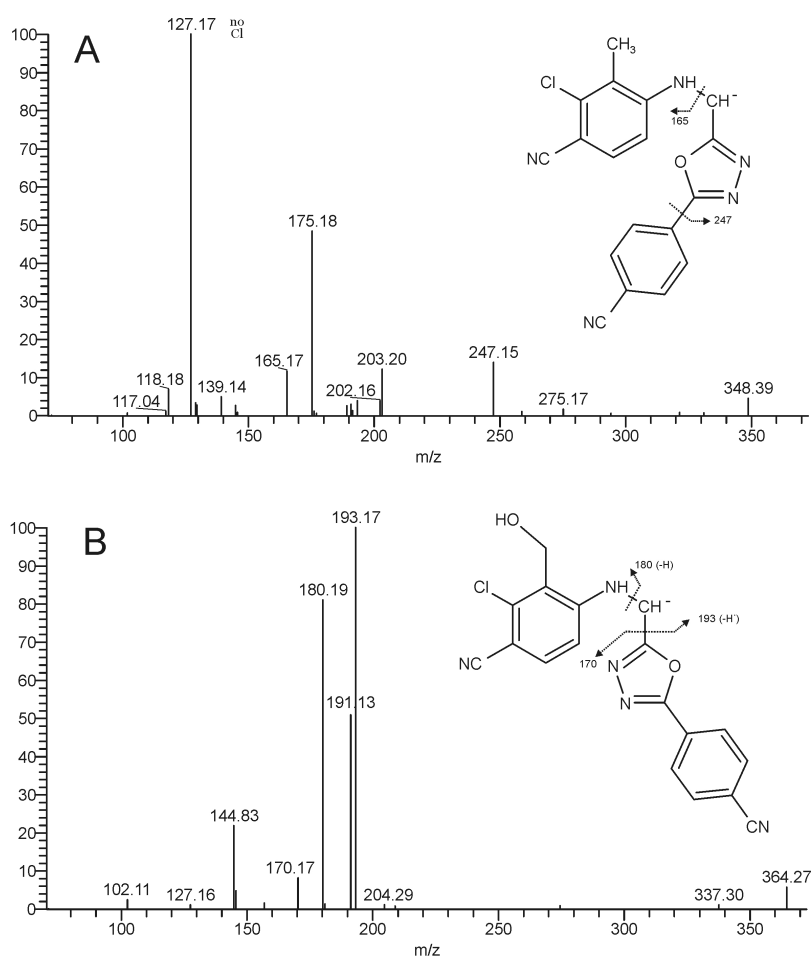


Fig. 2. Product ion negative ESI mass spectra of RAD140 (A) and its hydroxy metabolite (B). The ion formed due to the in-source elimination of acetaldehyde  $[M-CH_3CO-H]^-$  was used as a precursor in both cases.

After that we attempted an excretion study with RAD140 to compare the results. A healthy male volunteer took a single dose of 10 mg of this SARM after receiving the ethical approval from Russian Institute of Sport. The urine samples collected for 8 days (due to limited availability of the volunteer) were analyzed using the instrumental method created with the help of the *in vitro* data. It was found that only monohydroxy metabolites (two closely eluting isomers) were produced *in vivo*, with the parent compound being 10-20 times more abundant (Fig. 3).

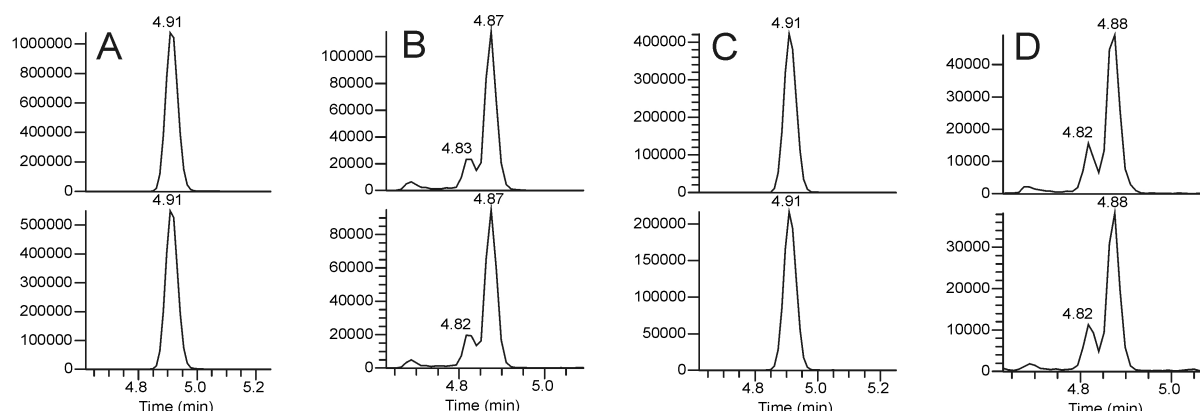


Fig. 3. Mass chromatograms demonstrating the detection of RAD140 (RT 4.91 min) and its hydroxy metabolites (RT 4.82, 4.88 min) at 22 h (A, B) and 8 days (C, D) after administration of 10 mg.

RAD140 and its hydroxy metabolites are almost completely conjugated with glucuronic acid. It is not clear whether these are O- or N-glucuronides. In the last urine sample RAD140 was still perfectly detectable, and therefore its detection period could be roughly estimated as 10-14 days. Table 1 lists the SRM transitions and respective collision energy used for the detection of RAD140 in urine.

Name	SRM (CE), negative ESI
RAD140	348 > 127 (25)
	348 > 175 (20)
hydroxy-RAD140	364 > 193 (20)
	364 > 180 (23)

Table 1. The SRM transitions for detection of RAD140 and its metabolite by LC-MS/MS

## Conclusions

Chromatographic and mass spectrometric properties of novel non-steroidal SARM RAD140 are presented. This compound was found to be resistant to metabolic reactions, both *in vitro* and *in vivo*, with only slight metabolism by hydroxylation. For doping control purposes the detection of the parent compound seems to be sufficient. It may be assumed that RAD140 can be detected up to 2 weeks after administration of a single dose of 10 mg.

## **References**

- [1] Miller CP, Shomali M, Lyttle CR, O'Dea LStL, Herendeen H, Gallacher K, Paquin D, Compton DR, Sahoo B, Kerrigan SA, Burge MS, Nickels M, Green JL, Katzenellenbogen JA, Tchesnokov A, Hattersley G. Design, synthesis, and preclinical characterization of the selective androgen receptor modulator (SARM) RAD140. *ACS Med. Chem. Lett.* 2011, 2, 124-129
- [2] Thevis M, Piper T, Beuck S, Geyer H, Schänzer W. Expanding sports drug testing assays: mass spectrometric characterization of the selective androgen receptor modulator drug candidates RAD140 and ACP-105. *Rapid Commun Mass Spectrom.* 2013, 27(11), 1173-1182