Title: A liquid chromatography–tandem mass spectrometry (LC-MS/MS) Method for the measurement of Testosterone Undecanoate and Dihydrotestosterone Undecanoate

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Abstract:

Background: An oral formulation of Testosterone Undecanoate (TU) is being developed for testosterone replacement in hypogonadal men. We need to assess the pharmacokinetics of the administered precursor testosterone undecanoate (TU) and its metabolite (DHTU) to assess the absorption and bioavailability in men.

Objective: We developed and validated LC-MS/MS method for the measurement of TU and DHTU in serum for Phase 2 pharmacokinetics of orally administered TU in men and applied the assay to measure the pharmacokinetics of TU and DHTU in serum after oral administration with food.

Method: Serial blood samples were collected after oral administration of TU 200 mg T equivalents at 8 hours in 26 men while fasting or after a meal. Blood for TU and DHTU were collected in tubes containing 30 mg of fluoride to prevent the dissociation of the undecanoate moiety to T and DHT. Deuterium-labeled d_{21}-TU and d_{21}-DHTU were used as internal standards (IS) and 50µL of serum sample processed by liquid/liquid extraction for TU and DHTU. The LC-MS/MS system used a Shimadzu high-performance LC 20 series system (Columbia, Maryland) with an Applied Biosystems API 5500 with ESI source (Foster City, California) operated in the positive ion detection mode using multiple reaction monitoring (MRM) of the transitions m/z .The parent/product ion for TU and DHTU were monitored at m/z 457.5/97.1 and m/z 459.4/255.2, respectively, and m/z 478.7/97.2 and m/z 480.7/255.2 for internal standards D21TU and D21DHTU. TU and DHTU were separated on a Thermo Hypersil GOLD LC-8 DB

column (50mm x 4.6 mm,5um) Waltham, Massachusetts) with a gradient profile at a flow rate of 0.4 mL/min with a mobile phase of MeOH and 98% H_2O (2% MeOH, 0.1% formic acid). The method was linear over a concentration range of 1 to 3000 ng/mL for TU and DHTU. The lower limit of quantification (LLOQ) is 1.0 ng/mL for TU and DHTU. The CV of intra-assay and inter-assay precision, and accuracy spanning the different concentrations were 7.9%, 7.6% to 10.4%, and 83.1% to 108.3% for TU; and 13.9%, 9.6% to 15%, and 97.6% to 110.4% for DHTU respectively.

Results: The pharmacokinetics over 24 hours of TU and DHTU after oral administration of TU (200mg of T equivalent) at 8 hours are shown in the table below.

Table 1. Pharmacokinetics Parameters of TU and DHTU after oral administration of TU 200 mg as T equivalents in men (mean±SEM)

	TU with Food		TU Fasting	
	TU (n=26)	DHTU (n=26)	TU (n=22)	DHTU (n=23)
AUC (ng/ml/12hr)	824±72.6	561±48.5	118±12.9	60±11.9
Cavg (ng/ml/hr)	68.7±6.0	46.8±4.0	9.8±1.1	5.0±1.0
Cmax (ng/ml)	218.2±30.6	103.7±9.6	29.7±3.6	11.1±1.6
Cmin(ng/ml)	5±2.0	5.1±1.8	1.0±0.02	1.3±0.2
Time to Cmax (h)	4.4±0.6	5.3±0.6	3.2±0.2	2.7±0.5

Serum TU and DHTU levels are lower if the TU was administered without food. When TU was administered with a meal, serum TU levels peaked at 4.4 hour which was followed by serum

DHTU levels. 12 hours after oral administrations serum TU and DHTU levels were no longer measurable.

Conclusion: We have developed and validated LC-MS/MS assays for TU and DHTU. We utilized the validated method for pharmacokinetics study of orally bioavailable testosterone ester (TU) and its metabolite (DHTU) for androgen replacement in testosterone deficient men.