

Supplementary Materials: Probing Steroidal Substrate Specificity of Cytochrome P450 BM3 Variants

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Table S1. Compounds used in this study.

No.	Steroid	Structure	Source
1	cholesterol	3-hydroxy- Δ^5 -steroid	Sigma-Aldrich, St. Louis, MO, USA
2	β -sitosterol	3-hydroxy- Δ^5 -steroid	National Institutes for Food and Drug Control, Beijing, China
3	diosgenin	3-hydroxy- Δ^5 -steroid	Sigma-Aldrich, St. Louis, MO, USA
4	ergosterol	3-hydroxy- Δ^5 -steroid	National Institutes for Food and Drug Control, Beijing, China
5	methyltestosterone	3-keto- Δ^4 -steroid	National Institutes for Food and Drug Control, Beijing, China
6	androstenediol	3-hydroxy- Δ^5 -steroid	Alke Reagent, Chengdu, China
7	testosterone	3-keto- Δ^4 -steroid	J&K Chemical Ltd., Shanghai, china
8	dehydroepiandrosterone	3-hydroxy- Δ^5 -steroid	Alke Reagent, Chengdu, China
9	androstenedione	3-keto- Δ^4 -steroid	National Institutes for Food and Drug Control, Beijing, China
10	pregnenolone	3-hydroxy- Δ^5 -steroid	National Institutes for Food and Drug Control, Beijing, China
11	progesterone	3-keto- Δ^4 -steroid	National Institutes for Food and Drug Control, Beijing, China
12	17 α -hydroxypregnenolone	3-hydroxy- Δ^5 -steroid	Sigma-Aldrich, St. Louis, MO, USA
13	17 α -hydroxyprogesterone	3-keto- Δ^4 -steroid	J&K Chemical Ltd., Shanghai, China

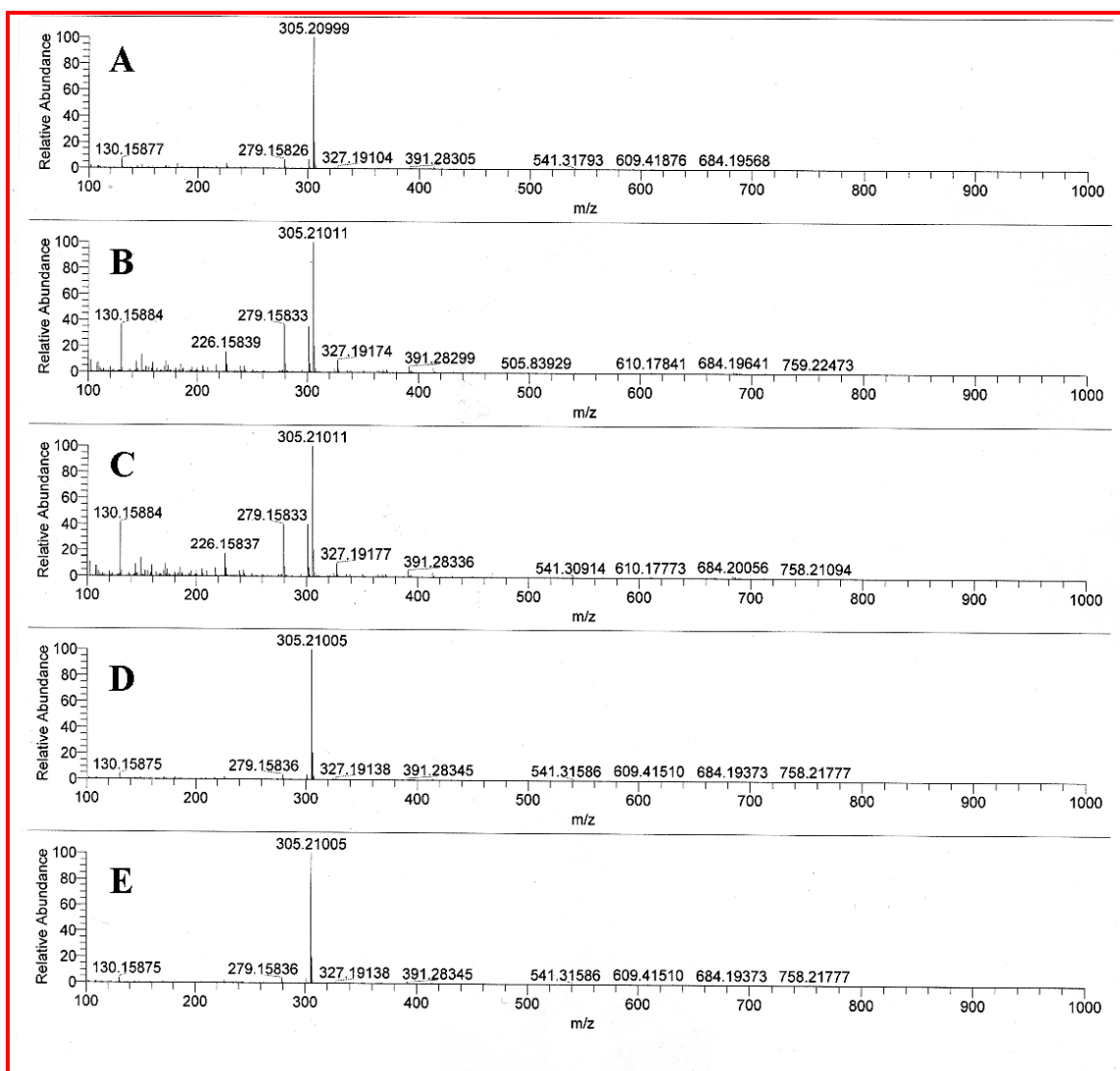


Figure S1. ESI mass spectra of metabolites 2 (A); 3 (B); 4 (C); 5 (D) and 6 (E) of testosterone produced by M01A82WS7L.

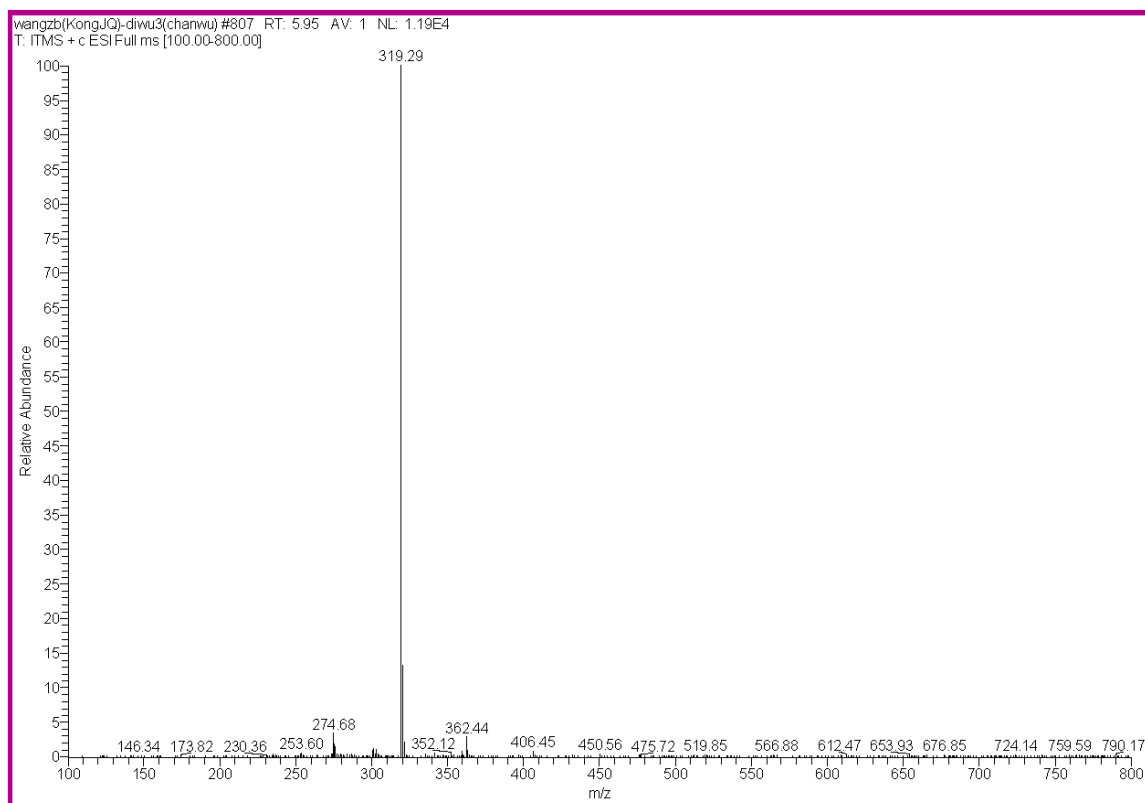


Figure S2. ESI mass spectra of 16 β -OH-MT (metabolites 2) produced by M01A82W.

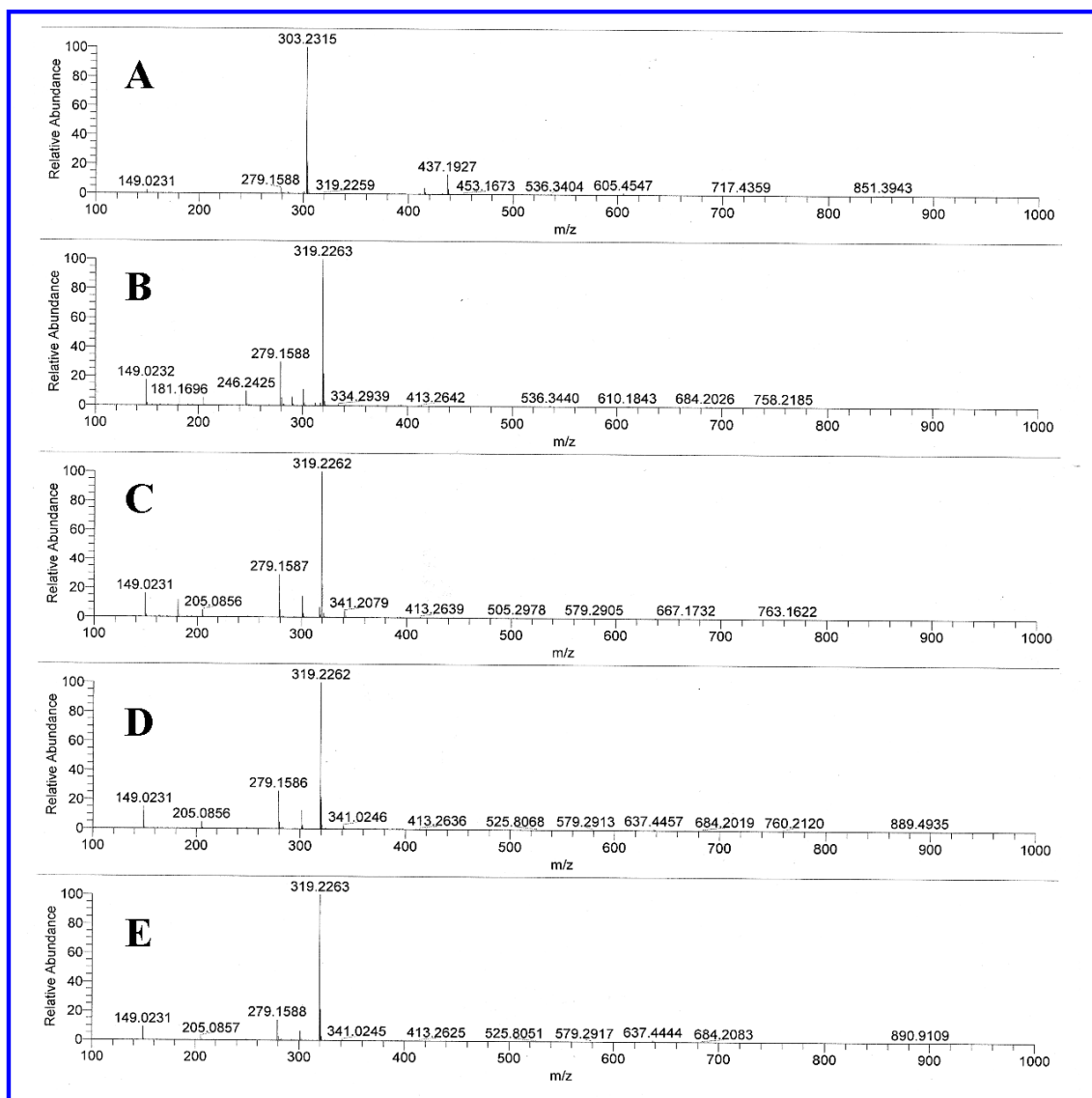


Figure S3. LC-MS analysis of methyltestosterone (A) and its monohydroxy metabolites (B-E) formed by P450 BM3 mutant pET28aS72I.

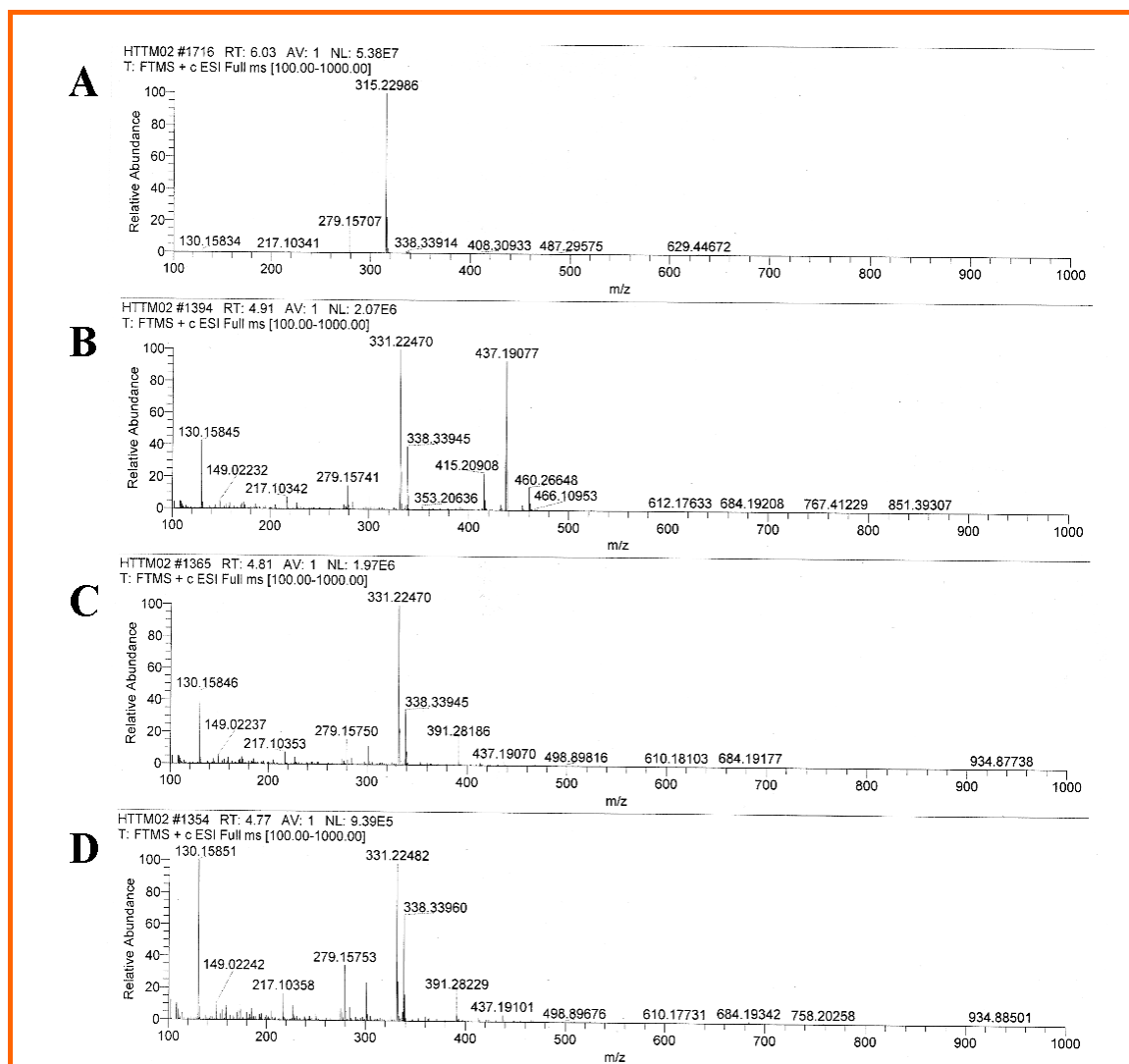


Figure S4. ESI mass spectra of progesterone (A) and its three monohydroxylated metabolites 2 (B); 3 (C) and 4 (D).

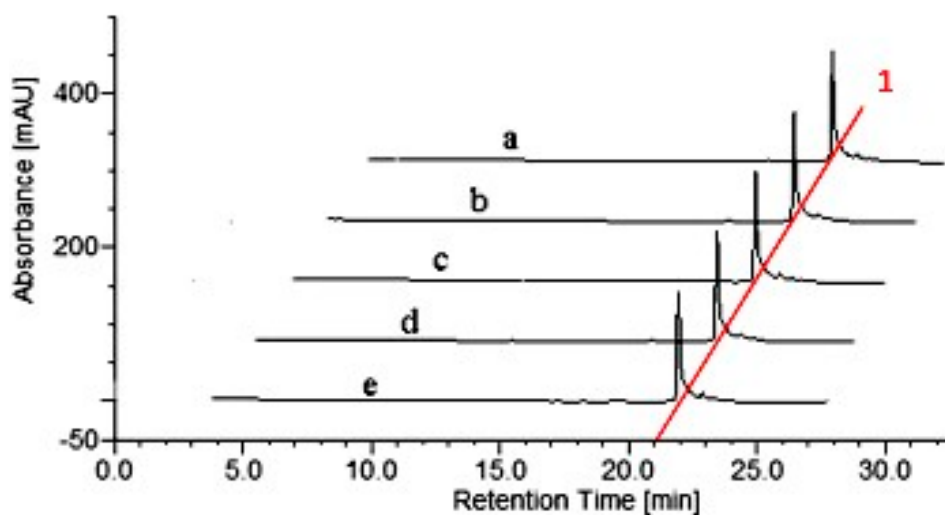


Figure S5. HPLC chromatograms of pregnenolone (a); pregnenolone incubations with empty vector (b); M01A82W (c); M01A82WS72I (d) and M11A82W (e). 1 shows pregnenolone standard.

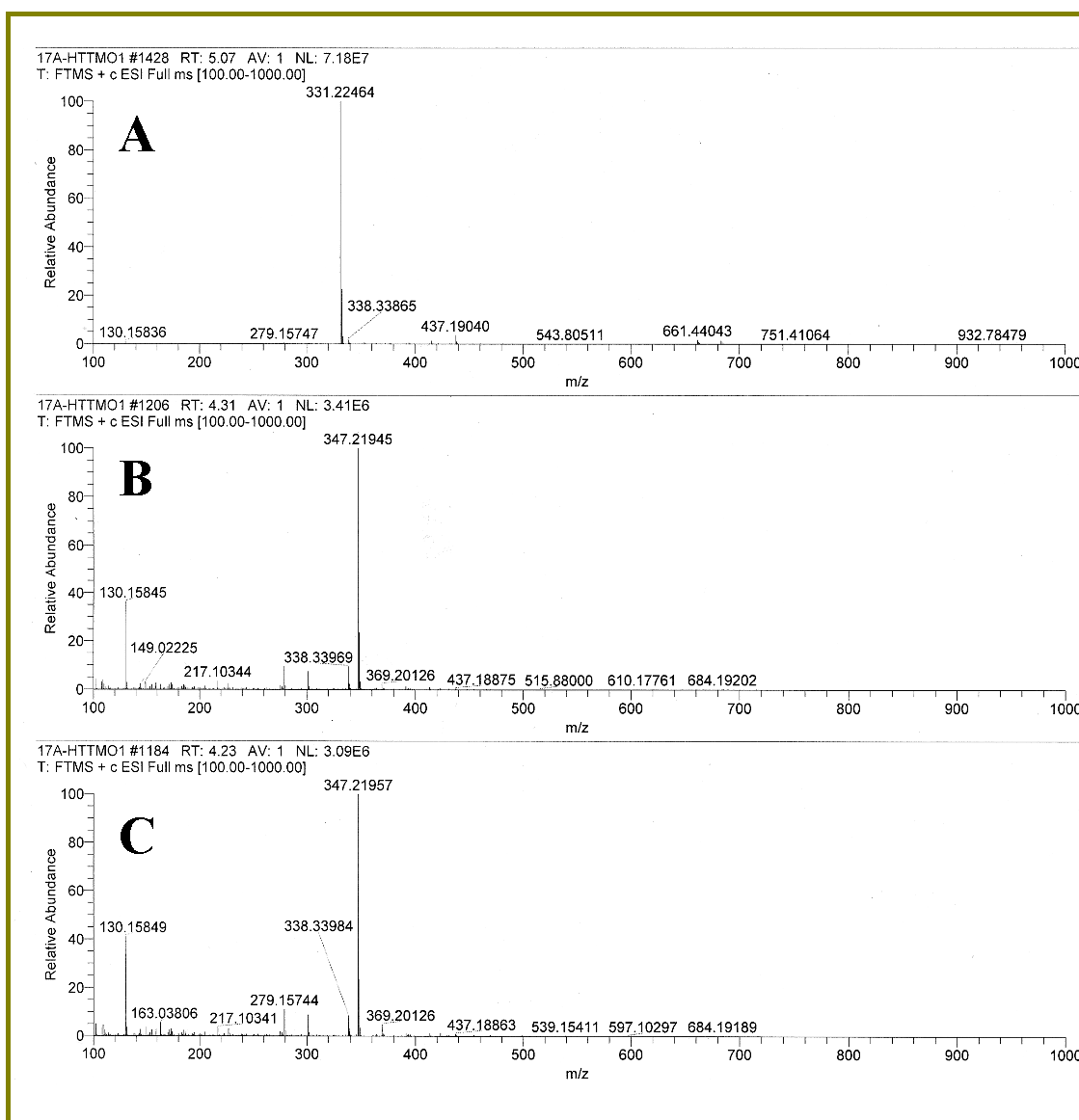


Figure S6. ESI mass spectra of 17 α -hydroxyprogesterone (A) and its two monohydroxylated products 2 (B) and 3 (C).

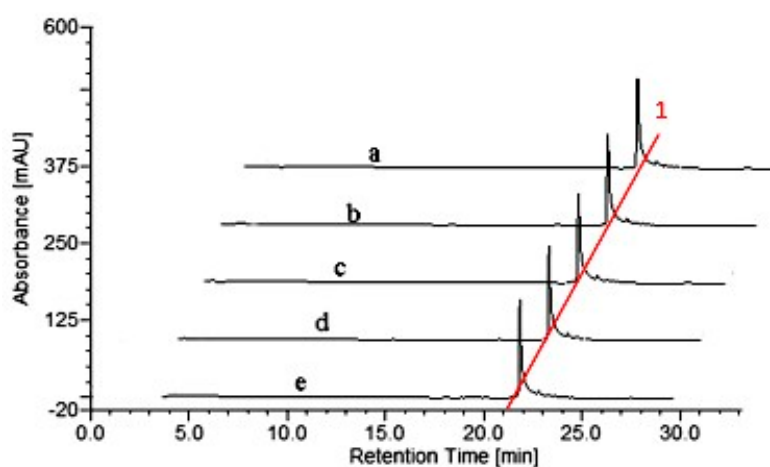


Figure S7. HPLC chromatograms of 17 α -hydroxypregnenolone (a); 17 α -hydroxypregnenolone incubations with empty vector (b); M01A82W (c); M01A82WS72I (d) and M11A82W (e). 1 shows 17 α -hydroxypregnenolone standard.

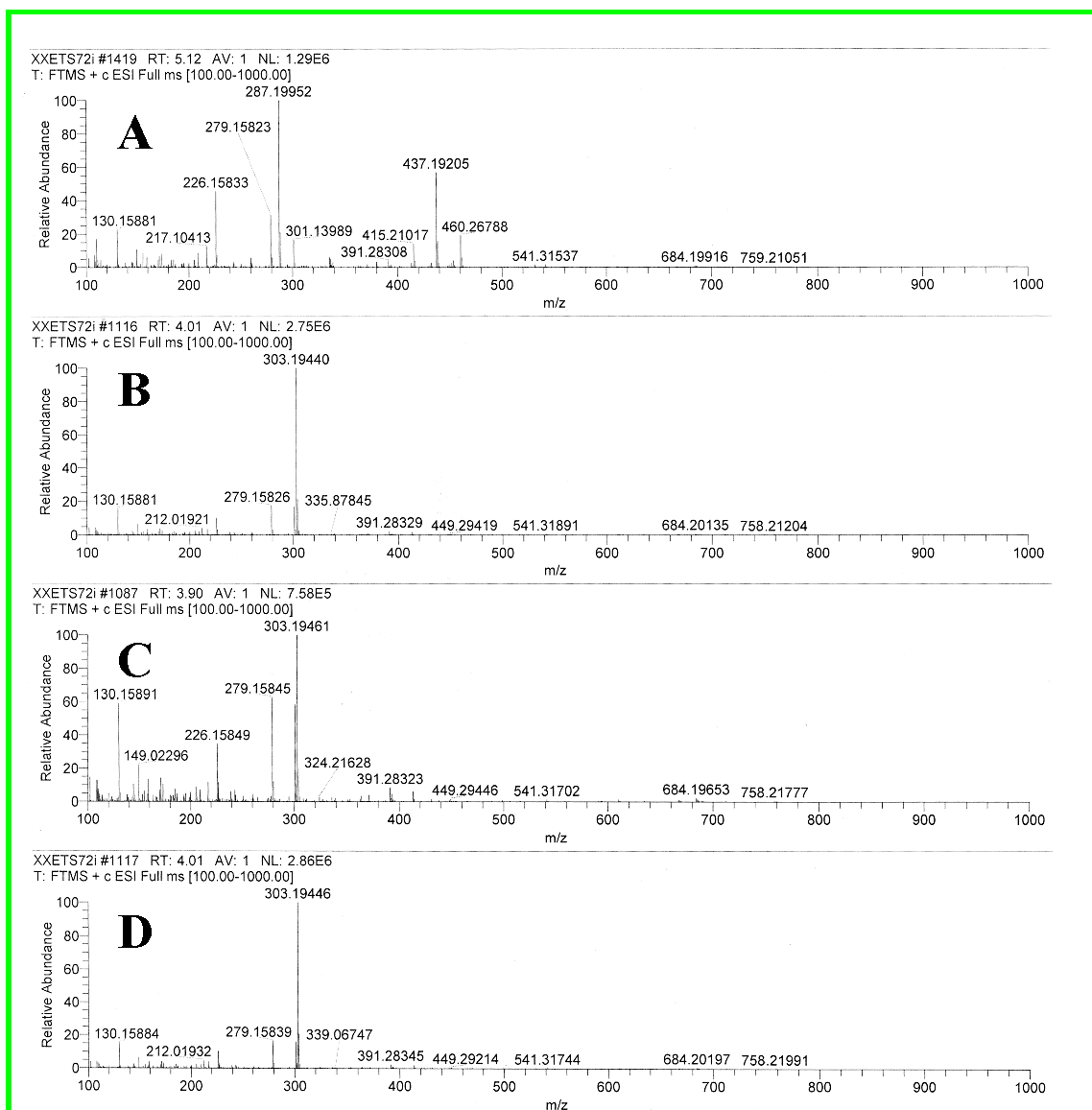


Figure S8. ESI mass spectra of androstenedione (A) and its three monohydroxylated metabolites 2 (B); 3 (C) and 4 (D).

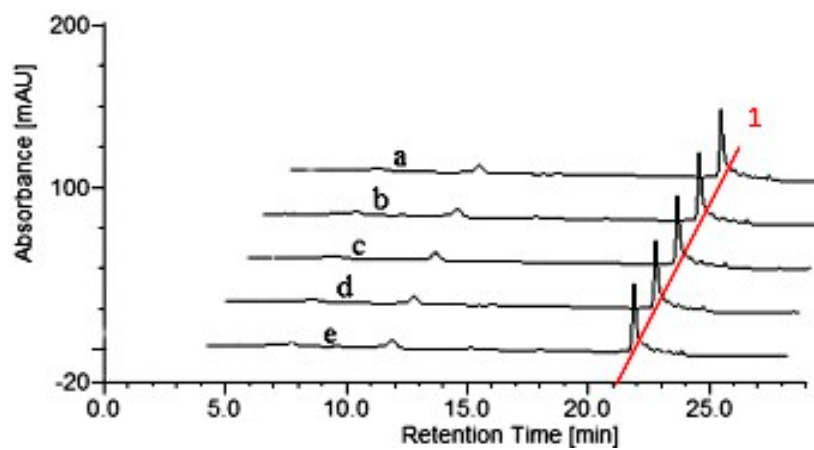


Figure S9. HPLC chromatograms of DHEA (a); DHEA incubations with empty vector (b); M01A82W (c); M01A82WS72I (d) and M11A82W (e). 1 shows DHEA standard.

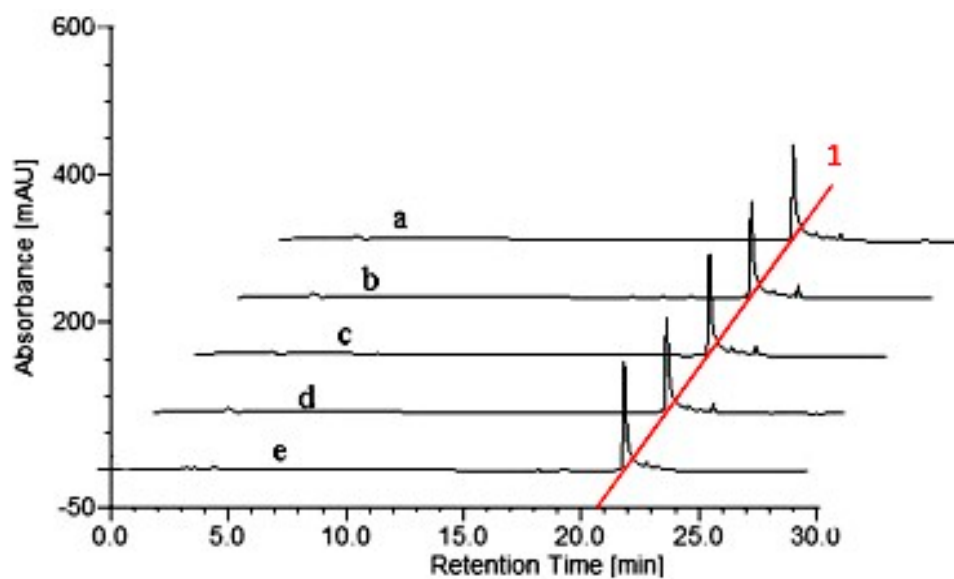


Figure S10. HPLC chromatograms of androstenediol (a); androstenediol incubations with empty vector (b); M01A82W (c); M01A82WS72I (d) and M11A82W (e). 1 shows androstenediol.