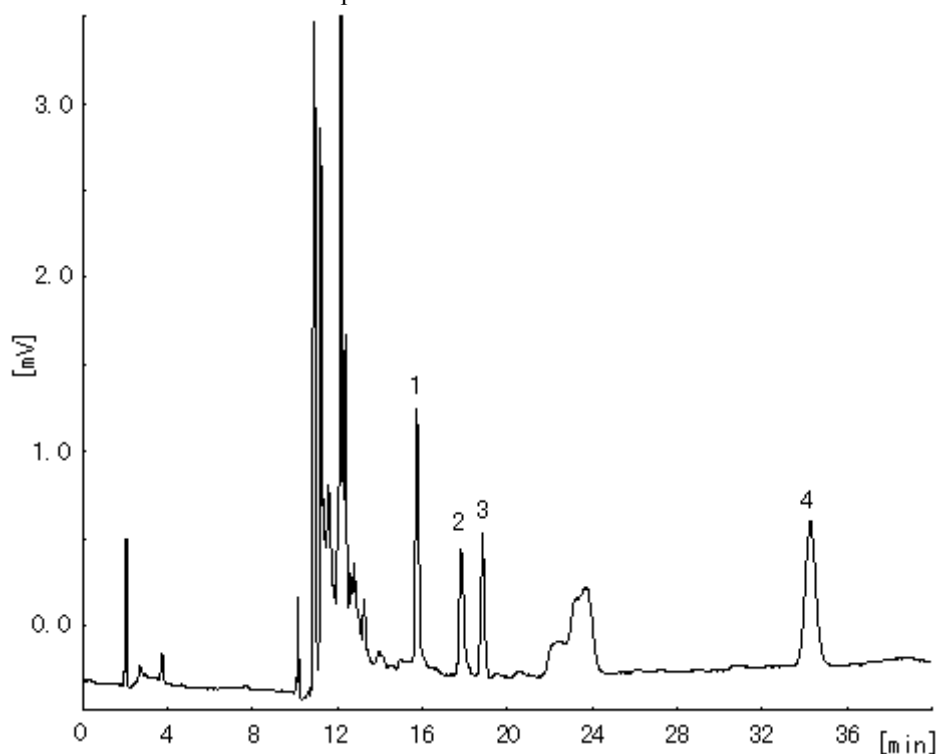


Steroids are added to calf bovine serum at 5pmol/L each.



1	Testosterone	2	Androstenedione	3	17 α -Hydroxyprogesterone
	5 pmol/L		5 pmol/L		5 pmol/L
4	Progesterone				
	5 pmol/L				

Conditions of Primary separation and Focusing

Precolumn	CAPCELL PAK C18 UG120 S5 : 2.0 mm i.d.×35 mm
Intermediate column	CAPCELL PAK MF Cartridge (Ph-1) : 4.0 mm i.d.×10 mm
Mobile phase	100mmol/L Phosphate buffer / CH ₃ CN = 90 / 10
Flow rate	500 μ L/min
Temperature	40 °C
Detect	UV
Inj. vol.	100 μ L

Conditions of Final separation

Analytical

column	CAPCELL PAK C ₁₈ UG120 S3 : 2.0 mm i.d.×100 mm
Mobile phase	100mmol/L Phosphate buffer / CH ₃ CN = 68 / 32
Flow rate	300 μL/min
Temperature	40 °C
Detect	UV 245 nm