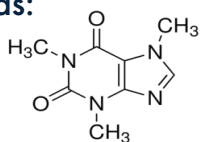
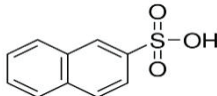


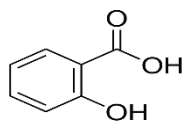
Compounds:



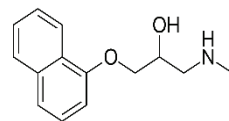
Caffeine



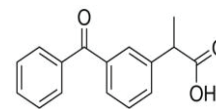
2-Naphthalenesulfonic acid



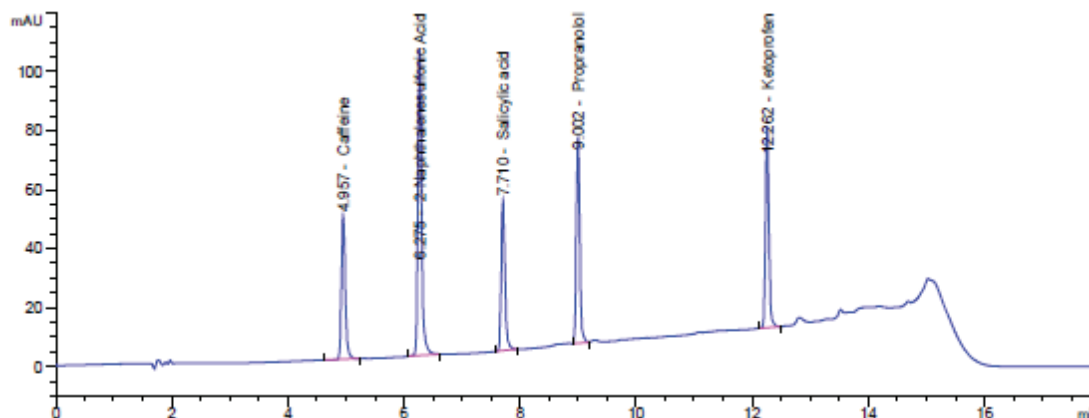
Salicylic acid



Propranolol



Ketoprofen



MicroElution Plate Extraction Method:

Agility Deluxe WAX micro elution, 3 mg/ well,
 Cat #OC03AGD7002

Condition

50 μ L of methanol, followed by 50 μ L of water

Load

50 μ L of sample in PBS (0.500 μ g caffeine, 0.125 μ g 2 naphthalenelfonic acid, 0.375 μ g salicylic acid, 0.175 μ g propranolol, and 0.750 μ g ketoprofen)

Wash

50 μ L 25 mM sodium acetate buffer pH = 4

Elute

Elute neutral and basic compounds (E1) with 50 μ L of methanol
 Elute acidic compounds (E2) with 50 μ L of 20% (v/v) ammonium hydroxide in 20/80 (v/v) methanol/acetonitrile

Dilute

Dilute E1 with 50 μ L of PBS Dilute E2 with 50 μ L of 20% (v/v) phosphoric acid in water

HPLC-UV Method

Column	Orosil C18 HC, 3 μ m
Column Size	4.6 mm ID x 150 mm long
Part Number	03FI-023
Temperature	40 $^{\circ}$ C
Mobile Phase Gradient	A: 20 mM Potassium Phosphate Monobasic in 10% MeOH, pH 3.0 B: MeOH Time B (%) 0 25 12 90 13 25 18 25
Injection	20 μ L
Flow Rate	0.8 mL/min
UV Wavelength	230 nm

Recovery and precision test results:

	Recovery%	CV(%)
Caffeine*	103.3	0.7
Propranolol*	105.1	1.4
2-Naphthalenesulfonic Acid	110.3	5.5
Salicylic Acid	112.0	5.5
Ketoprofen	109.9	5.3