

## **Universal HPLC-UV Method for Complex Mixtures**

Column:	Primesep 200	1	Uracil (PN)
Part number:	200-46.150.0510	2	Epinephrine (PB)
Column size:	4.6 x 150mm, 5 um, 100A	3	DOPA (PB)
Mobile phase:	A: 5% ACN with 0.05% $H_2SO_4$ B: 80% ACN with 0.25% $H_2SO_4$ From 100% A to 50% A in 10 min, then to 25% A in 6 minutes	4	2,6-Lutidine (PB)
		5	Benzylamine (PB)
Flow rate:	1.0 ml/min	6	Hydroxytryptophan (PB)
Detection:	215 nm	7	Homovanillic acid (PA)
	15	8	Phenol (PN)
6	14	9	Tryptophan (PB)
	10 12 9 11	10	2,3-DHBA (PA)
2 3		11	Benzoic acid (PA)
5 7 1 4		12	Methylparaben (HN)
		13	Ethylparaben (HN)
		14	Toluene (HN)
0 5	10 15 min	15	Amitriptyline (HB)

## **Application Comments**

Analytical chemists face multiple complex separations everyday. Very often, complex mixtures containing various compounds need to be analyzed in a single run. Traditional reversed-phase chromatography has challenges for retention of polar-neutral, polar-acidic and polar-basic compounds in mixtures with hydrophobic compounds. We have developed a universal screening method for analysis of complex mixtures containing polar-neutral, polar-basic, polar-acidic, hydrophobic-neutral and hydrophobic-basic compounds.

The method employs Primesep 200 mixed-mode reversed-phase cation-exchange column and a simple mobile phase containing ACN/water/sulfuric acid. The low pH of the mobile phase helps suppress ionization of polar-acidic compounds, making them slightly hydrophobic. Hydrophobic and hydrophilic neutral compounds are retained by RP mechanism, while basic hydrophilic and basic hydrophobic compounds are retained by cation-exchange mechanism.

Abbreviations: PN-polar neutral, PB-polar basic, PA-polar acidic, HN-hydrophobic neutral, HB-hydrophobic basic