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A development and application study for anion exchange + cation exchange + normal phase + reversed-phase multi-mode ODS column

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SUMMARY

There are a lot of hydrophilic compounds which are related to pharmacology, food, and biochemistry, including: water soluble vitamins, catecholamines, organic acids, nucleotides, etc. These compounds are difficult to retain by reversed-phase analysis without the use ion-pairing additives in the mobile phase.

Several mixed-mode reversed-phase stationary phases consisting of alkyl and ion exchange ligands have been introduced into the HPLC column market **(Fig-1)**. However, these mixed-mode stationary phases have the following disadvantages: (1) Column designs that employ either RP/cation or RP/anion offer limited solutions for unknown samples (difficult to choose which column to use) and (2) The RP/anion/cation phase columns that are marketed today consist of only one ligand structure. A balance between hydrophobicity and ionic strength cannot be controlled when this surface structure is employed.

We have overcome these disadvantages with a revolutionary multi-mode ODS phase structure. This technology consists of three types of ligands, including: ODS, anion, and cation ligands **(Fig-2)**. This mode of separation is advantageous for various types of analysis, including: hydrophilic anions, cations, and drug metabolite analysis containing both ionic-hydrophobic and hydrophilic compounds. In addition, it can be useful for LC-MS by using low buffer concentration without ion pairing additives.

RESULTS AND DISCUTTION

Fig-3

Scherzo SM-C18 is a multi-mode ODS column that provides the following modes of separation: reversed-phase, anion exchange, and cation exchange. These interactions enable the separation of water-soluble vitamins without the use of ion-pairing reagents.

Fig-4

Strong ionic compounds, such as quaternary amines or sulfonic acids, can be difficult to retain / separate on conventional ODS. Mixed-mode RP columns have a single ionic ligand (anion or cation) and struggle to retain both acidic and basic compounds. Separation of both acids and bases require two different methods with two different mixed-mode RP columns. In contrast, the multi-mode ODS column, Scherzo SM-C18, consists of both anionic and cationic ligands. Separation of both cations and anions is possible using one column and one method.

Fig-5

Because Scherzo SM-C18 is a multi-mode column consisting of ODS+cation+anion ligands, retention for ionic compounds can be affected by eluent pH.

Strong ionic solutes like sulfonic acid and quaternary amine have poor retention on conventional ODS column (regardless of eluent pH). However, retention for these ionic compounds on Scherzo SM-C18 (IEX) is dependent upon eluent pH (due to changes in ionic interaction). The data shows that retention for acidic compounds is highest using low pH conditions, highest for basic compounds using high pH conditions, and constant for neutral compounds (regardless of eluent pH).

Fig-6

One of the benefits to using Scherzo SM-C18 is its ability to retain ionic compounds. Elution for ionic compounds is dependent upon many parameters.

The left figure shows the relationship between organic solvent concentration and retention. The substituted benzene rings have the following properties: base (quaternary amine), acid (sulfonic acid), and neutral (amide). Each solute has unique ionic properties. As a result, elution for these ionic compounds changes with decreasing ionic strength (due to increasing organic solvent composition).

The middle figure shows retention vs. salt concentration. Benzamide (neutral) is unaffected by salt concentration. However, retention for the ionic compounds is affected by salt concentration.

The right figure shows retention vs. temperature. These three solutes have hydrophobic properties and therefore show decreasing retention with increasing temperature.

Fig-7

Hydrophobic interaction is an important interaction for the ODS phase. Scherzo SM-C18 has similar hydrophobicity as conventional ODS column. The right figure shows the relationship between retention and alkyl carbon number (n) of alkylbenzenes. The slope (log k / CH2) indicates hydrophobicity; the data shows hydrophobicity for Scherzo SM-C18 is similar to that of Unison UK-C18. Therefore, using SM-C18 and UK-C18 (under the same experimental condtions) may be useful for tracking elution behavior of ionic compounds.

The left figure shows similar results for separation of isomers on SM-C18 and UK-C18. This indicates that molecular recognition of hydrophobic compounds on SM-C18 is the same as conventional ODS columns. This excellent performance is observed on all 3um particle products (including multi-mode ODS).

Fig-8

Scherzo SM-C18 retains strong ionic compounds. The left figure shows that glycophosphates (low pKa values) interact strongly with cation ligands on the stationary phase. These compounds are retained under 100% aqueous conditions. Highly polar compounds, which are retained via ionic interaction (and do not interact with non-polar ligands), can also elute under normal phase conditions.

PNPG contains a phenol group and shows decreasing retention with increasing organic solvent. In contrast, sugar phosphates (highly polar anionic compounds) seem to exhibit normal phase behavior (the middle figure). This can be useful for LC-MS applications where the addition organic solvent improves ionization efficiency.

PNPG shows decreasing retention at elevated temperatures due to hydrophobicity. In contrast, sugar phosphates show no loss in retention at elevated temperatures (the right figure). Therefore, column temperature can be tuned to provide a balanced separation between polar and hydrophobic compounds.

Fig-9

The left figure shows the relationship between acetonitrile concentration and retention on Scherzo SM-C18. Tocopherol is a non-polar compound and requires high organic solvent composition for elution. Ibuprofen, an acidic and middle-polar compound, also shows reversed-phase elution. Caffeine is polar, but does have some hydrophobicity; retention is reduced as acetonitrile is increased up to 60% (RP), but increases as acetonitrile goes past 80% (NP). Ascorbic acid is highly polar; retention is reduced as acetonitrile is increased up to 40% (RP), but increases as acetonitrile goes past 50% (NP). Serine is highly polar (zwitter ion) and is difficult to retain on conventional ODS. Retention on SM-C18 increases over 50% organic (NP). Low polarity compounds are retained on SM-C18 via reversed-phase mode. In contrast, polar compounds may be retained via normal phase mode.

Vitamin formulation often includes both Vitamin C and Vitamin E. The polarity of these compounds is quite different and difficult to analyze with one method. The right figure shows both compounds are separated by using reversed-phase + normal phase. As mentioned above, tocopherol elutes via reversed-phase, but ascorbic acid is retained via normal phase at high organic composition. Therefore, separation is possible under isocratic elution with optimized eluent composition. This method will be useful for vitamin C and E analysis at quality control laboratories.

Fig-10

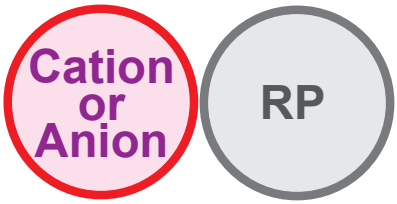
Mixed-mode RP columns struggle to achieve solute retention and repeatable separations as the interactions are complicated due to the presence of both reversed-phase and ion exchange modes. Scherzo SM-C18 addresses this problem with a novel stationary phase design to provides excellent reproducibility.

Fig-11 to 28

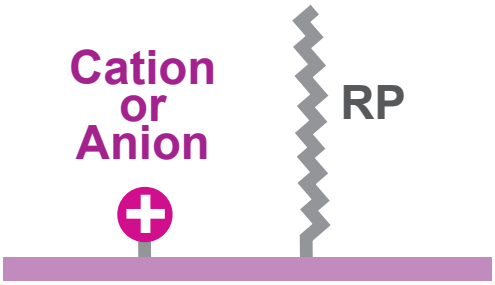
There are many difficult compounds to separate on conventional ODS column. Scherzo SM-C18 can retain or separate these compounds with individually optimized elution condition (pH, ionic strength etc.).

1 Mixed-Mode Reversed-Phase

Blend packing



Mixed bonding



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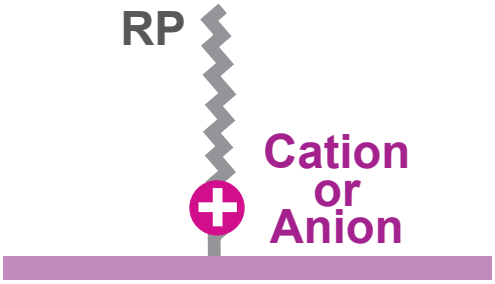
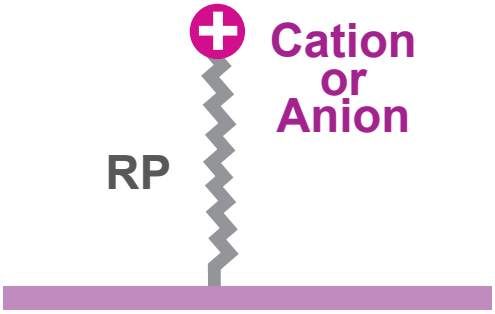
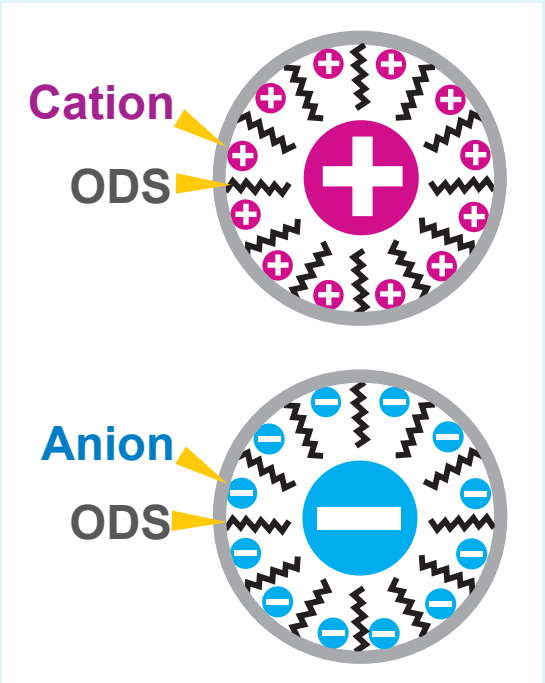


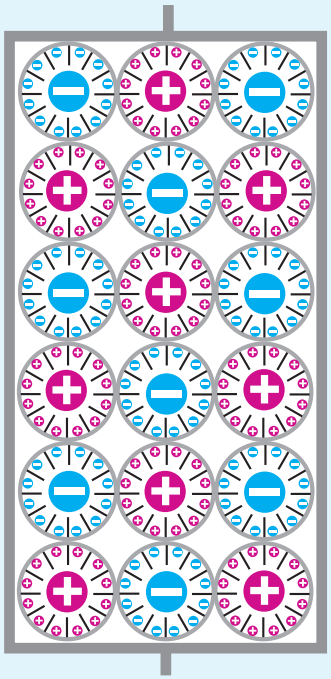
Tipped



2 Multi-Mode ODS Column Structure

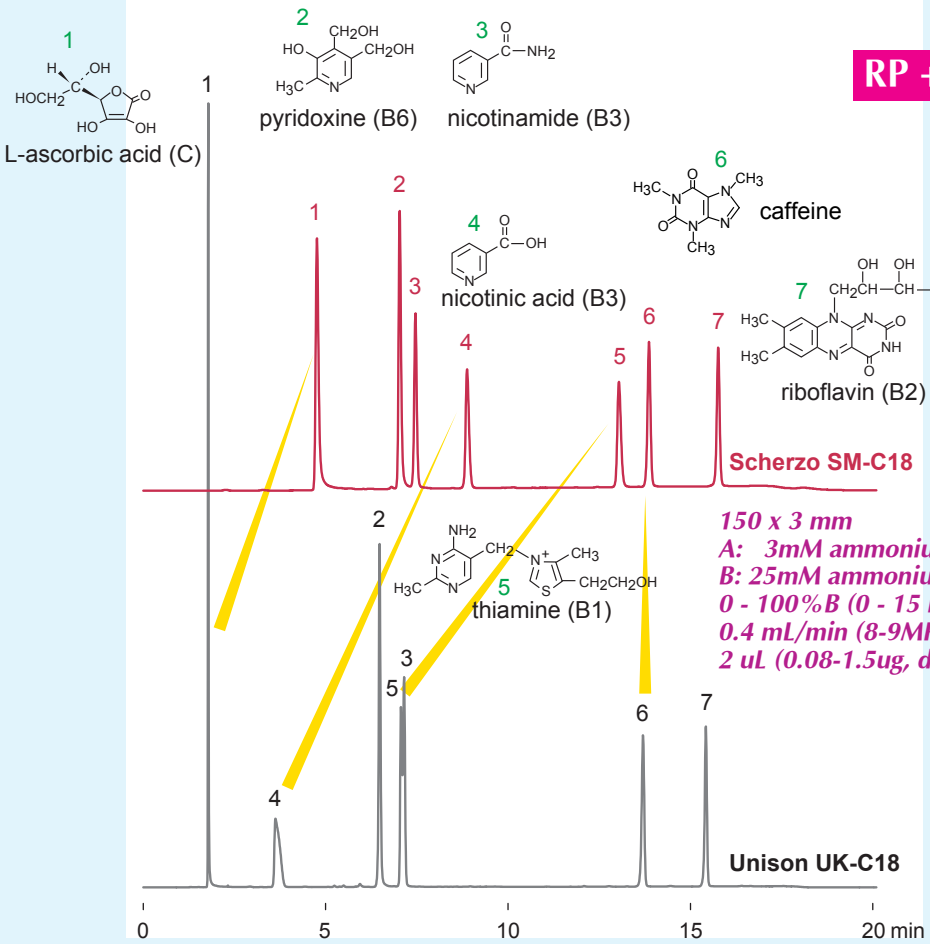
Scherzo (スケルツォ) SM-C18





Mixed bonding + Blend packing

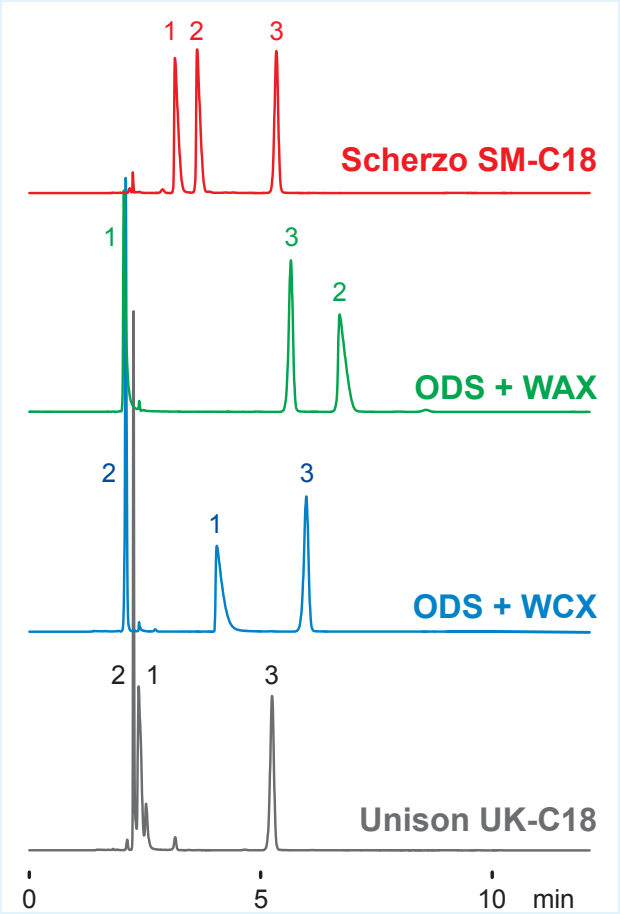
3 Water-soluble vitamins



RP + Anion Ex. + Cation Ex.

150 x 3 mm
A: 3mM ammonium formate
B: 25mM ammonium formate /acetonitrile = 80 / 20
0 - 100%B (0 - 15 min)
0.4 mL/min (8-9MPa), 37 deg.C, 260 nm
2 uL (0.08-1.5ug, diluted with 0.1M ammonium formate)

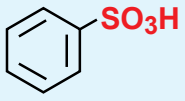
4 Comparison among ODS columns



1

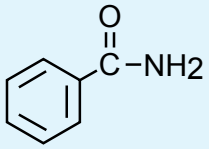
phenyltrimethylammnonium

2



benzenesulfonic acid

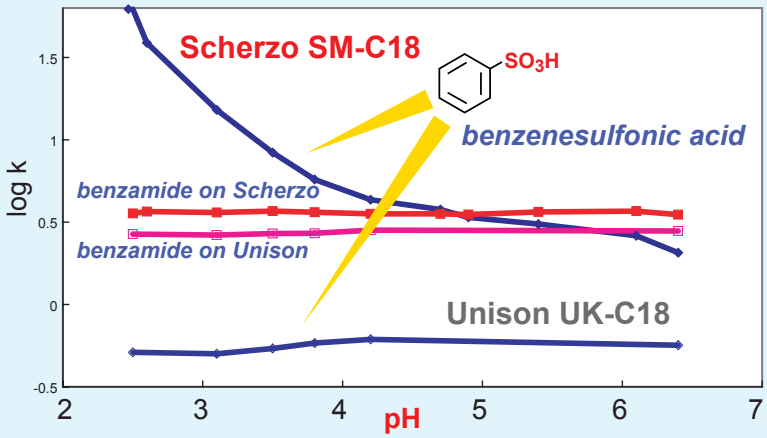
3

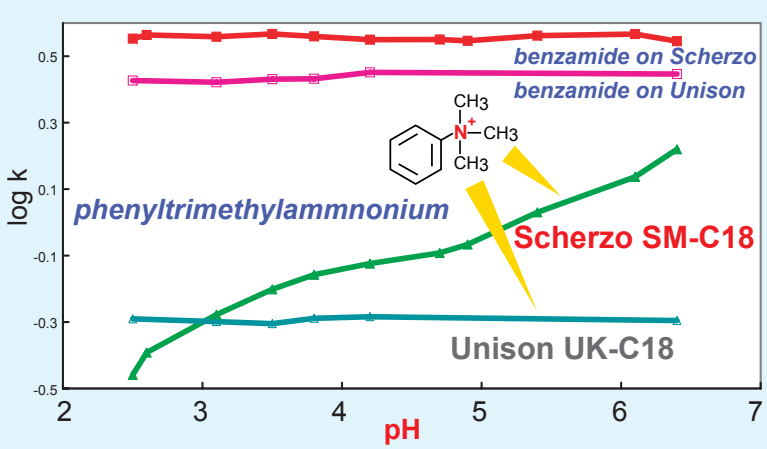


benzamide

150 x 3 mm
50 mM ammonium formate /acetonitrile = 85 /15
0.4 mL/min (8-9MPa), 40 deg.C, 260nm

5 pH dependency to log k



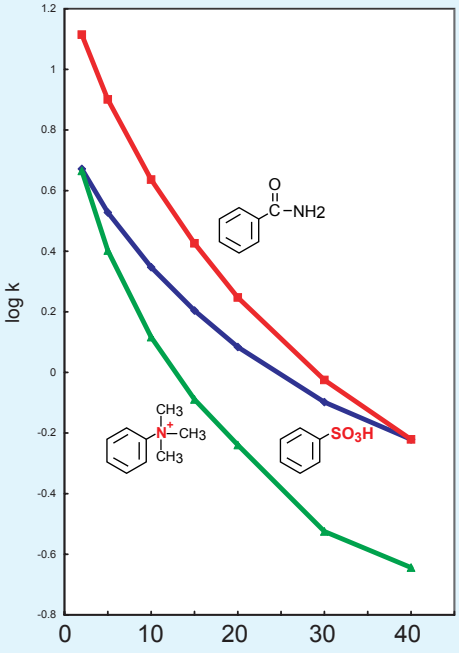


(50mM HCOOH - 50mM HCOONH4) /acetonitrile = 85 /15, 40 deg.C

6 Solvent, Ionic Strength, Temp Effect

RP + Anion Ex. + Cation Ex.

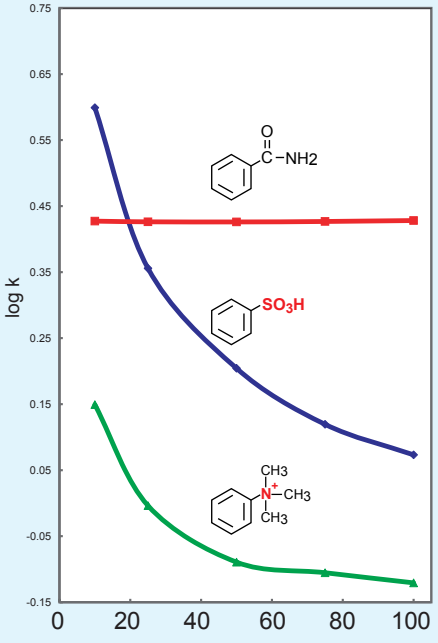
Organic Solvent



% acetonitrile

50mM HCOONH4 / ACN
40 °C

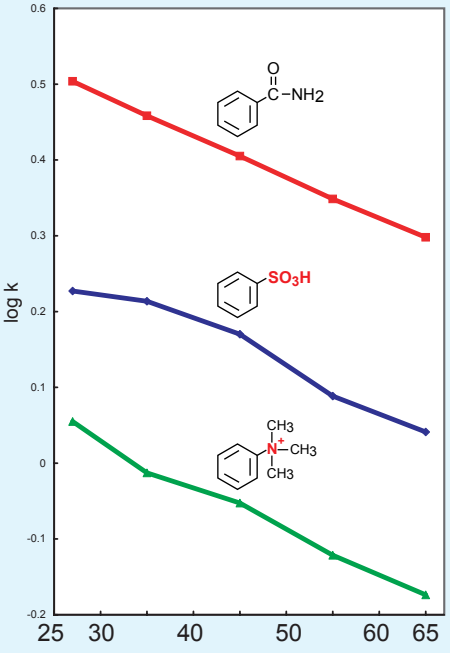
Ionic Strength



mM HCOONH4

HCOONH4 / ACN = 85 / 15
40 °C

Temperature

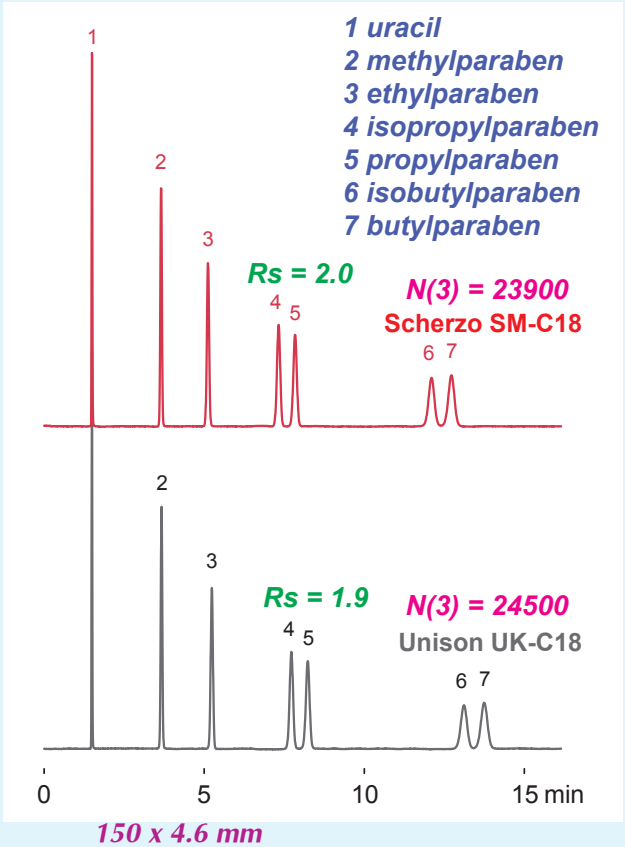


temperature (°C)

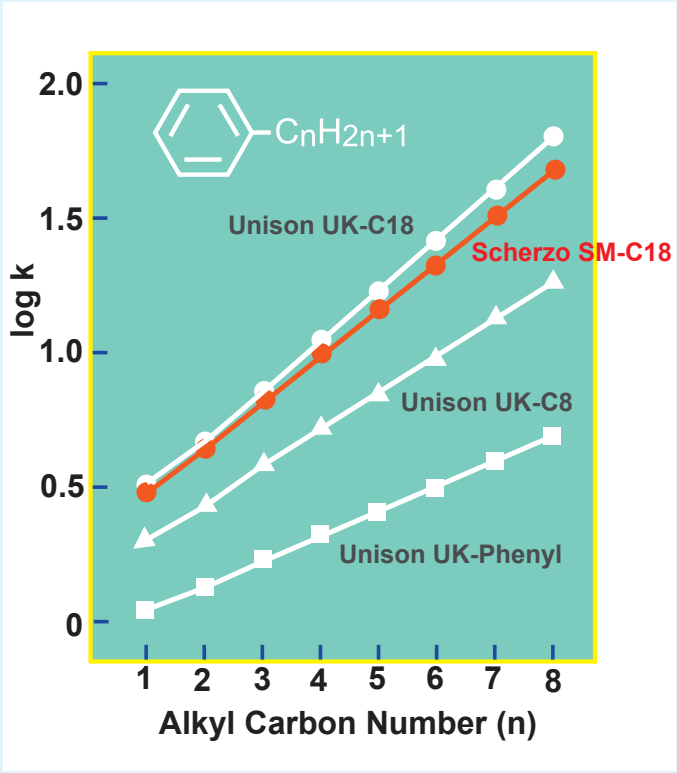
50mM HCOONH4 /ACN = 85 /15

7 Reversed-Phase Mode

RP



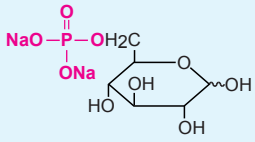
150 x 4.6 mm
water /ACN /AcOH = 60 /40 /0.1
1mL/min (10-11MPa), 37 °C, 260nm



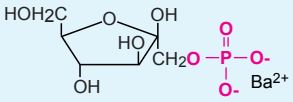
water /acetonitrile = 40 /60, 1 mL/min, 37 °C

8 Ion Exchange Mode

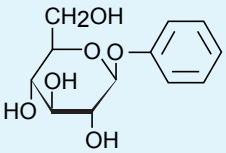
Anion Ex.



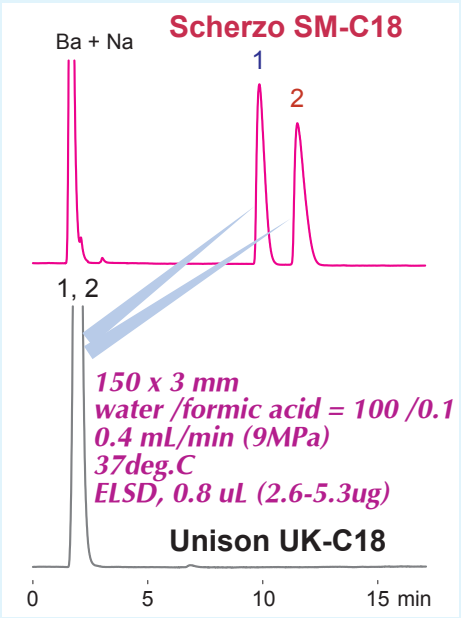
1) D- glucose-6-phosphate (G-6-P)



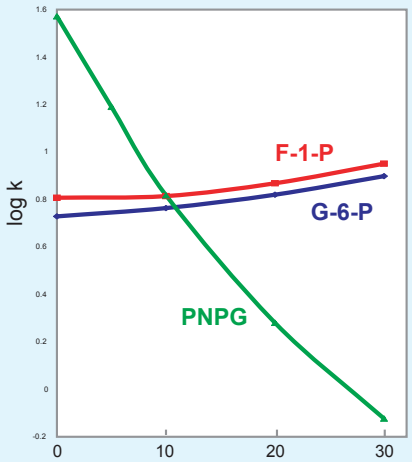
2) D- furctose-1-phosphate (F-1-P)



p-nitrophenyl-beta-glucoside (PNPG)

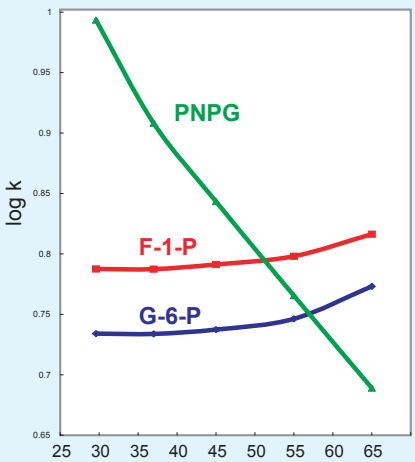


150 x 3 mm
water /formic acid = 100 /0.1
0.4 mL/min (9MPa)
37deg.C
ELSD, 0.8 uL (2.6-5.3ug)



% acetonitrile

water /ACN /HCOOH = A /B /0.1
37°C

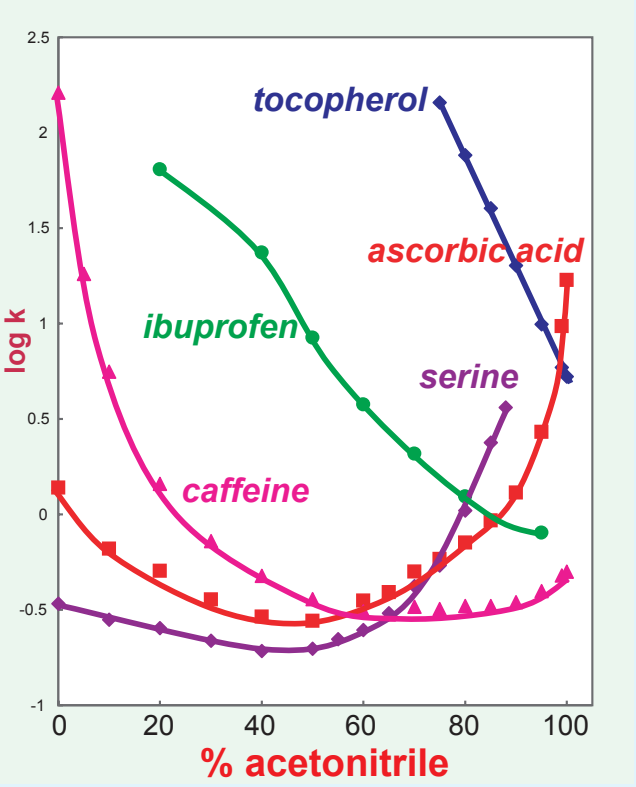


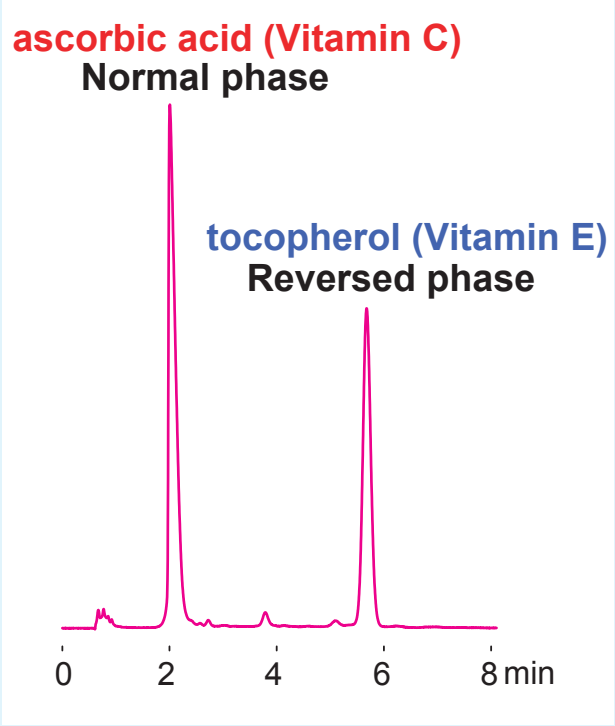
temperature (°C)

water /ACN /HCOOH = 93 /7 /0.1

9 Normal Phase Mode

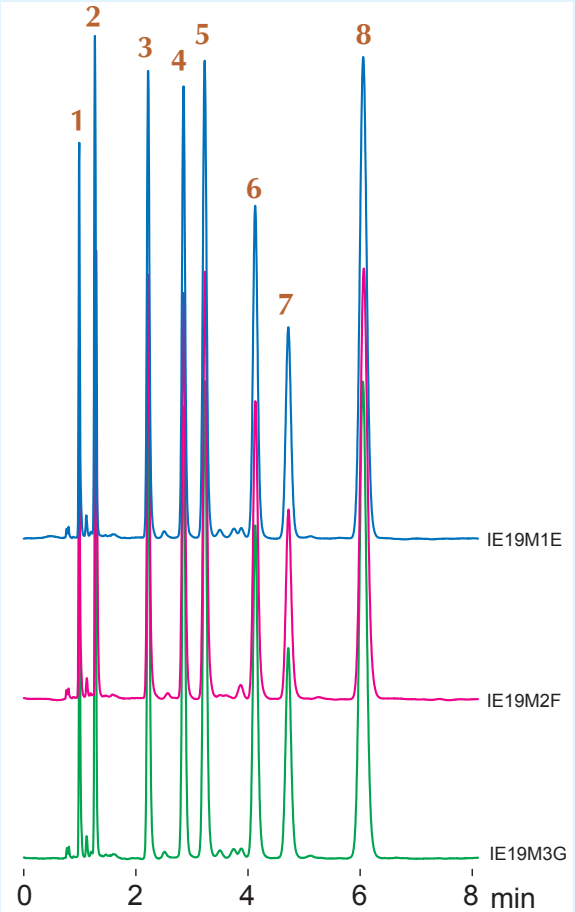
NP





Scherzo SM-C18, 50 x 3 mm
water /acetonitrile /formic acid = 5 /95 /0.1
0.4 mL/min, 37 °C, 270 nm

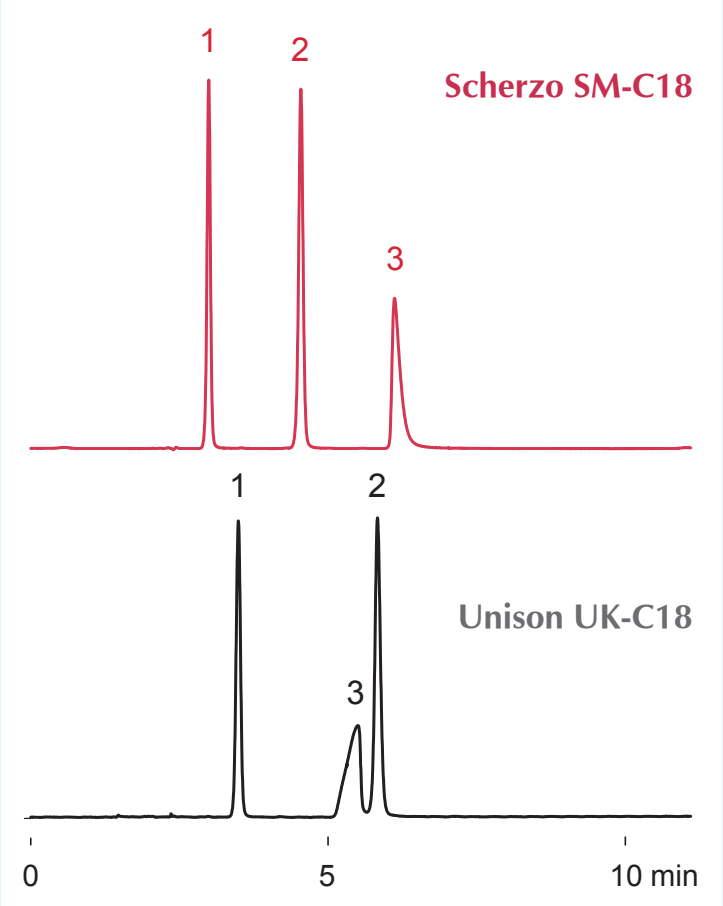
10 Batch-to-batch reproducibility

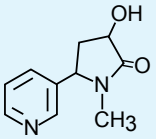


1) acetaminophen
2) 1-hydroxy-7-azabenzotriazole
3) prednisolone
4) methyl 3-amino-2-thiophenecarboxylate
5) 6-alpha-methylprednisolone
6) corticosterone
7) 4-aminobenzophenone
8) propylparaben

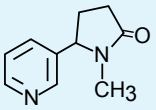
Scherzo SM-C18, 75 x 4.6mm
10mM ammonium acetate / ACN = 65 / 35
1mL/min, 37°C, 254nm

11 Nicotine

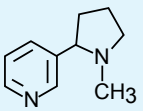




1) hydroxycotinine



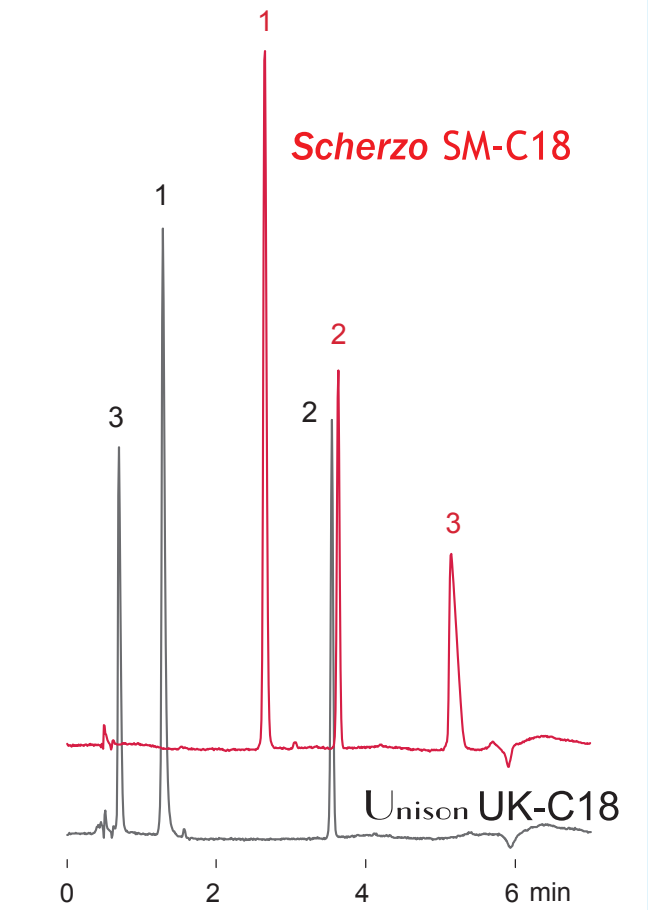
2) cotinine



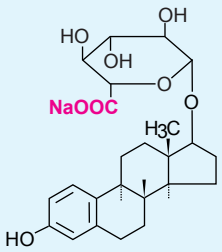
3) nicotine

150 x 3 mm
50mM ammonium acetate /ACN = 85 /15
0.4mL/min (9-10MPa), 37deg.C, 260nm
0.4uL (0.25ug)

12 Steroid hormone and metabolites

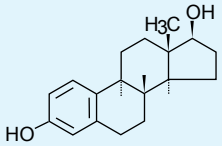


1)



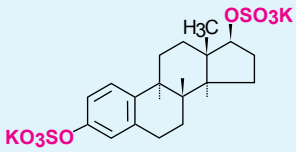
b-estradiol 17-(b-D-glucuronide)

2)



17b-estradiol

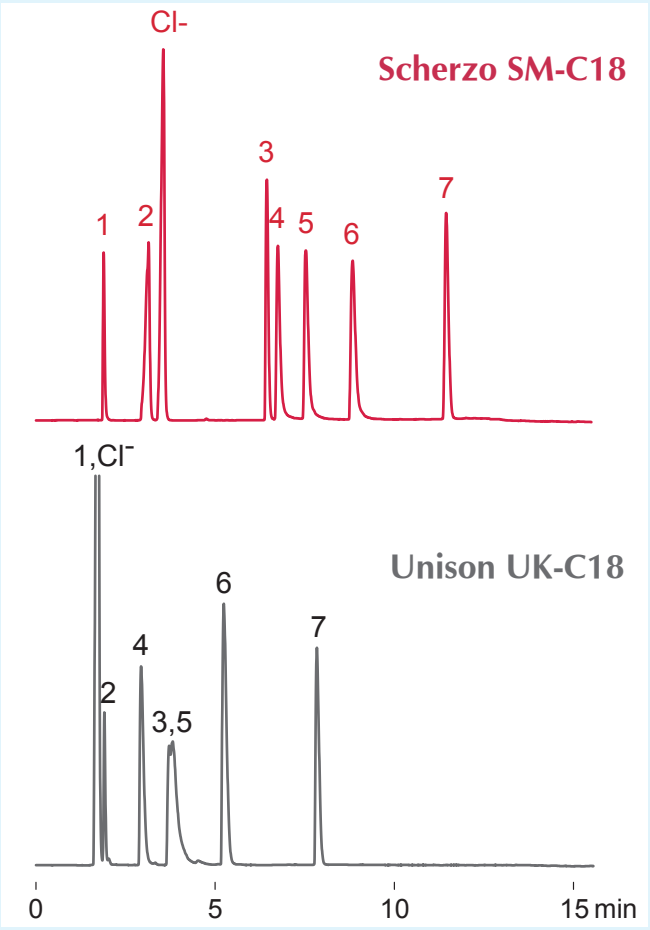
3)



b-estradiol 3,17-disulfate

50 x 3 mm
A: 5mM ammonium acetate
B: 125mM ammonium acetate /acetonitrile = 20 /80
30-100%B (0-5min)
0.5mL/min (5-9MPa), 37deg.C, 280nm
1uL (0.5-2.5ug)

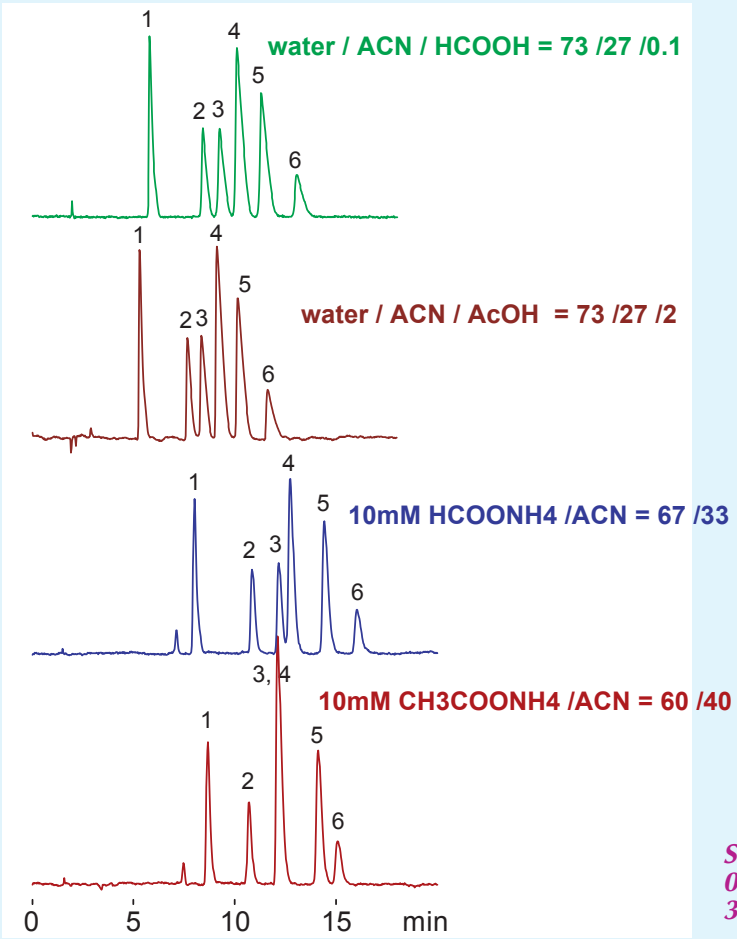
13 Neurotransmitters



1) 4-aminobutyric acid (GABA)
2) glutamic acid
3) acetylcholine hydrochloride
4) noradrenaline
5) adrenaline
6) dopamine hydrochloride
7) serotonin hydrochloride

150 x 3 mm
A: 3mM ammonium acetate
B: 80mM ammonium acetate /ACN = 80 /20
0-100%B (0-12min)
0.4mL/min (9MPa), 37deg.C, ELSD
3uL (0.65-2.6ug)

14 Basic compounds: Antidepressant drugs



1 doxepine pKa = 9.0

2 desipramine pKa = 10.4

3 imipramine pKa = 9.5

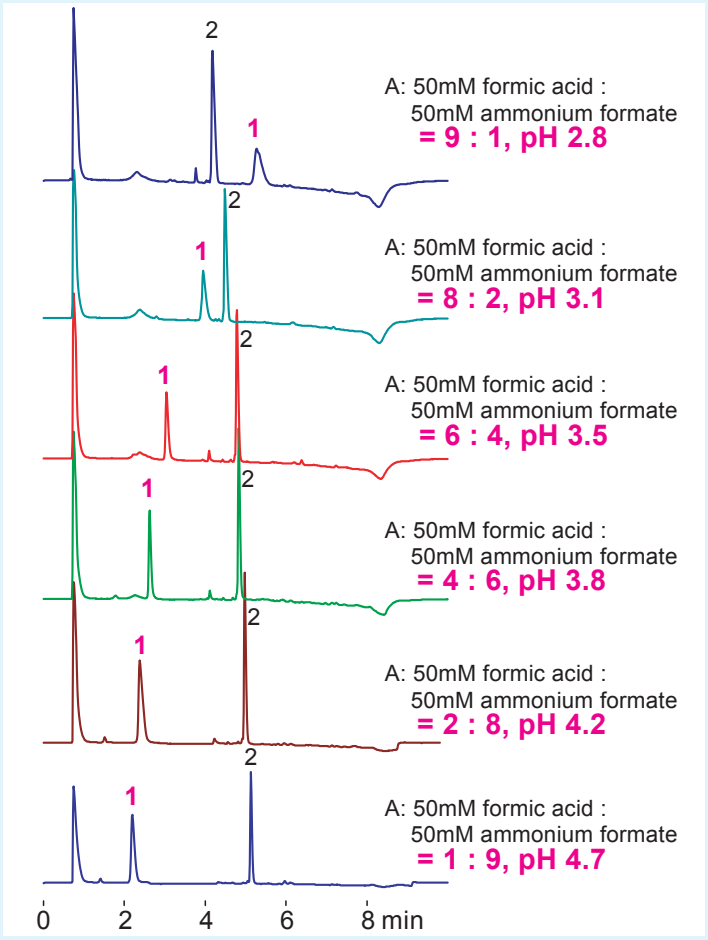
4 nortriptyline pKa = 9.7

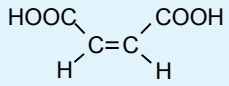
5 amitriptyline pKa = 9.4

6 trimipramine pKa = 8.0

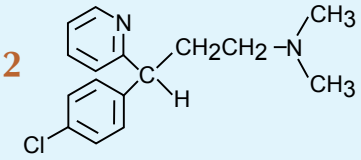
Scherzo SM-C18, 150 x 3 mm
0.4 mL/min (9-11MPa)
37 deg.C, 240 nm, 0.6 uL (120ng)

15 Basic Compound - salt



1 

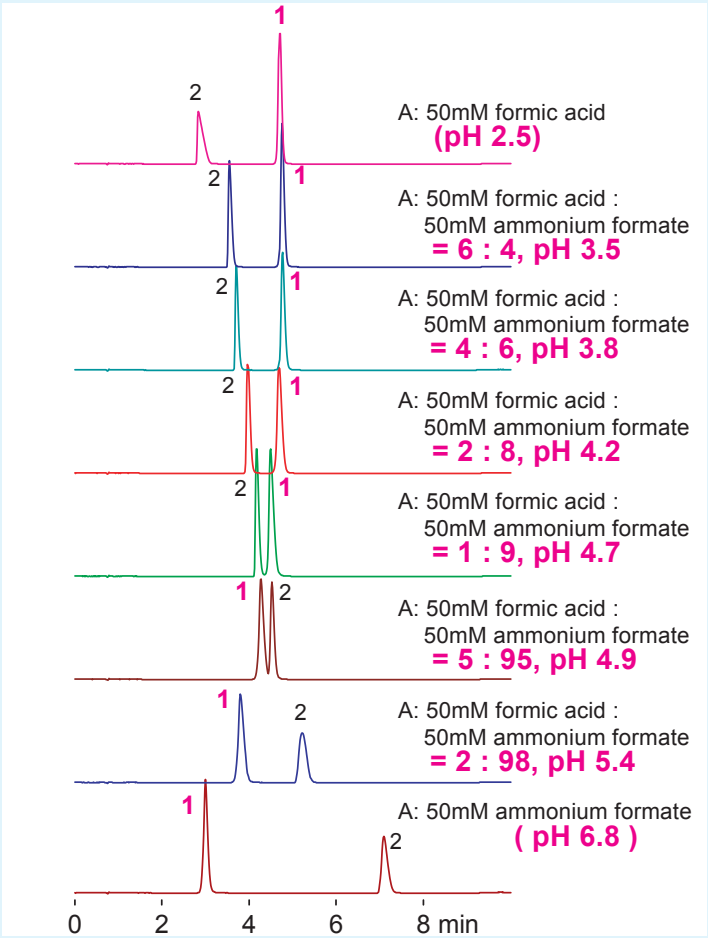
maleic acid

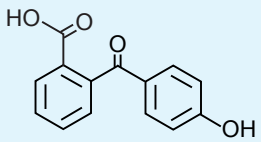
2 

chlorpheniramine

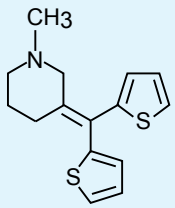
Scherzo SM-C18, 50 x 3 mm
A: formate buffer
B: acetonitrile
0-70 %B (0-7min)
0.4 mL/min (5MPa)
37 deg.C, 240 nm
2 uL (2ug)

16 Basic Compound - salt



1 

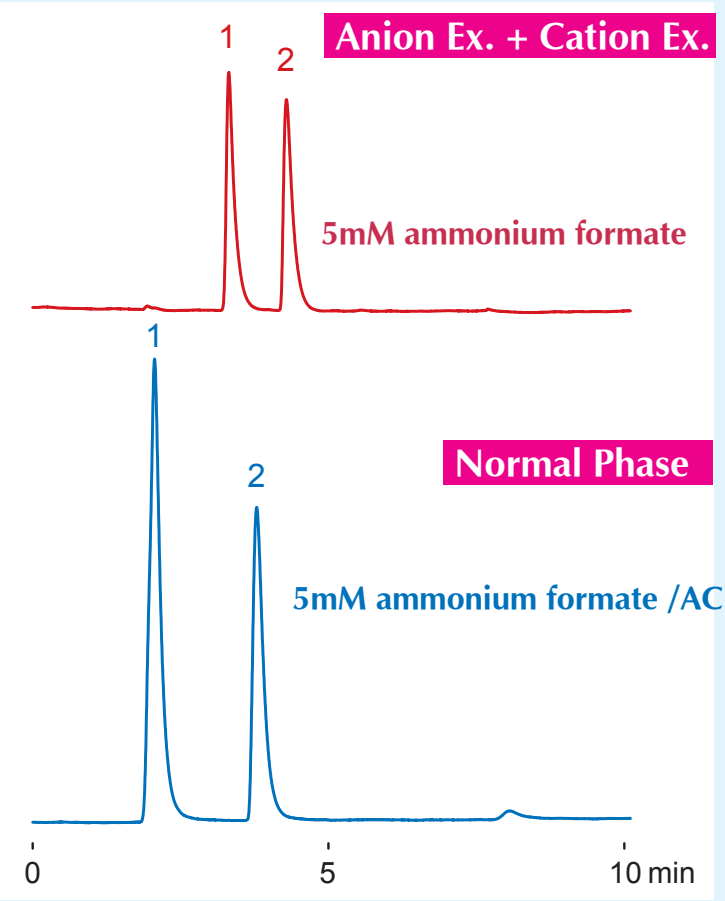
hibenzic acid

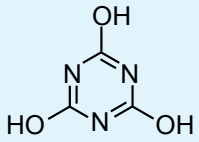
2 

tipepidine

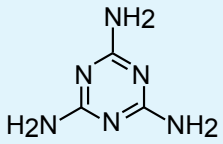
Scherzo SM-C18, 50 x 3 mm
A: formate buffer
B: acetonitrile
20-60 %B (0-8min)
0.4 mL/min (6-8MPa)
37 deg.C, 280 nm
0.6 uL (1.2ug)

17 Melamine / Cyanuric Acid - salt





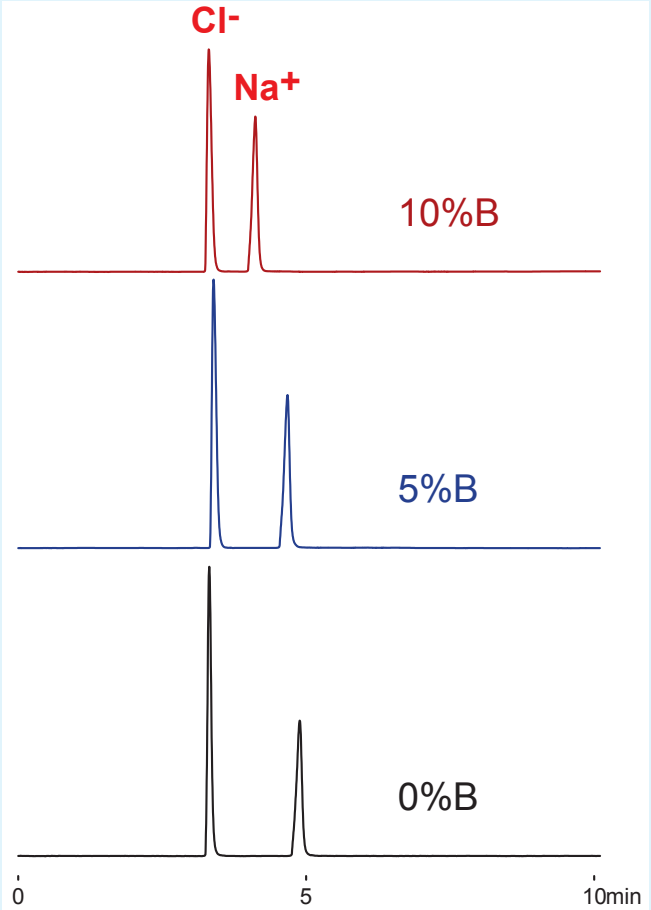
1) cyanuric acid



2) melamine

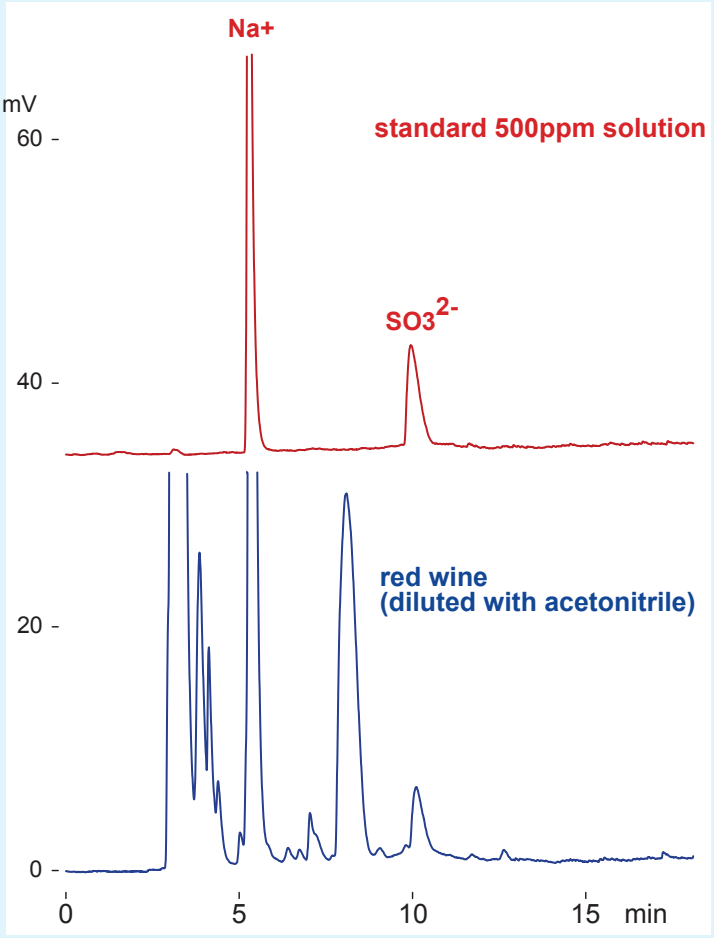
Scherzo SM-C18, 150 x 3 mm
0.4mL/min (4-9MPa), 37deg.C, ELSD
3uL (1.5ug, 2.5%NH4OH)

18 NaCl salt



Scherzo SM-C18, 150 x 3 mm
A: 5mM ammonium formate
B: acetonitrile
0.4 mL/min (9-10 MPa)
37 deg.C
ELSD (spray chamber 20 deg.C,
drift tube 45 deg.C)
1.6 uL (0.16ug NaCl)

19 Sodium Sulfite - salt



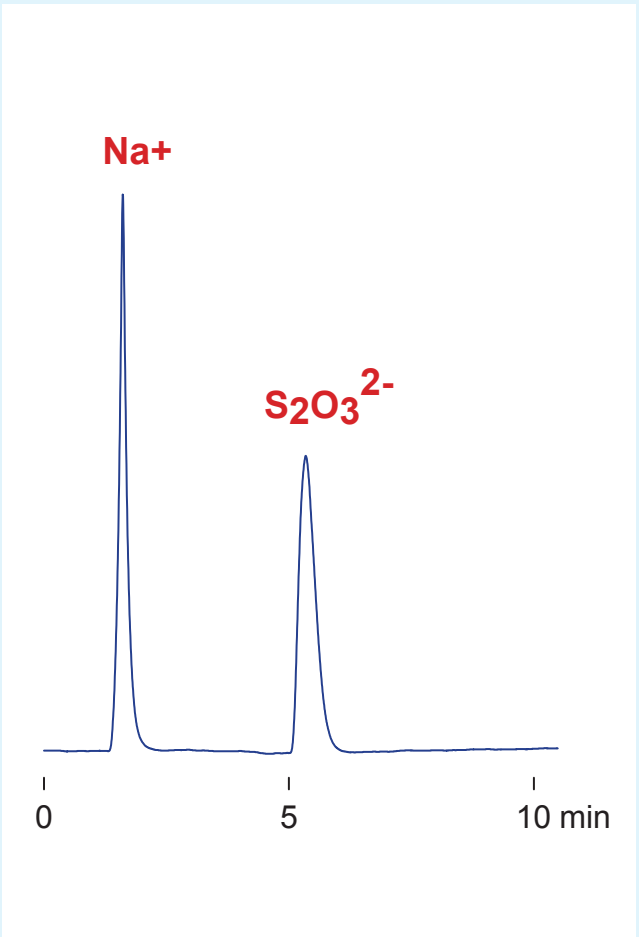
Na_2SO_3

sodium sulfite

Scherzo SM-C18, 250 x 3 mm
A: 5mM ammnoium acetate,
B: 100mM ammonium acetate /ACN = 50 / 50
10-100 %B (0-15min)
0.4 mL/min (14MPa), 37 deg.C
ELSD (spray chamber 20 deg.C,
drift tube 45 deg.C), 2 uL

541

20 Thiosulfate (H_2S metabolite)



$Na_2S_2O_3$

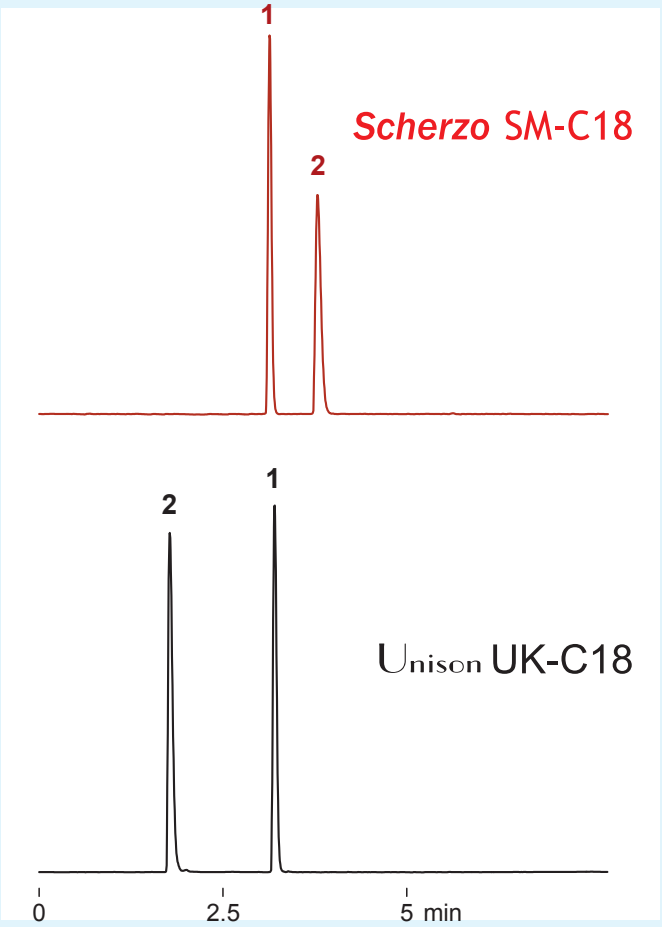
sodium thiosulfate

(H_2S metabolite)

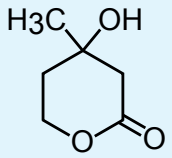
Scherzo SM-C18, 150 x 3 mm
A: 50 mM acetic acid /ACN = 90 /10
B: 100 mM ammoium acetate /ACN = 90 /10
0-100%B (0-3min), 100%B (3-10min)
0.4 mL/min (9MPa), 37 deg.C,
ELSD (spray chamber 50 deg.C,
drift tube 100 deg.C)
1.6 uL (16 ug)

549

21 Mevalonic Acid

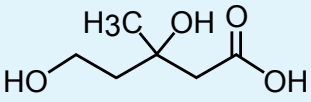


1



mevalonolactone

2

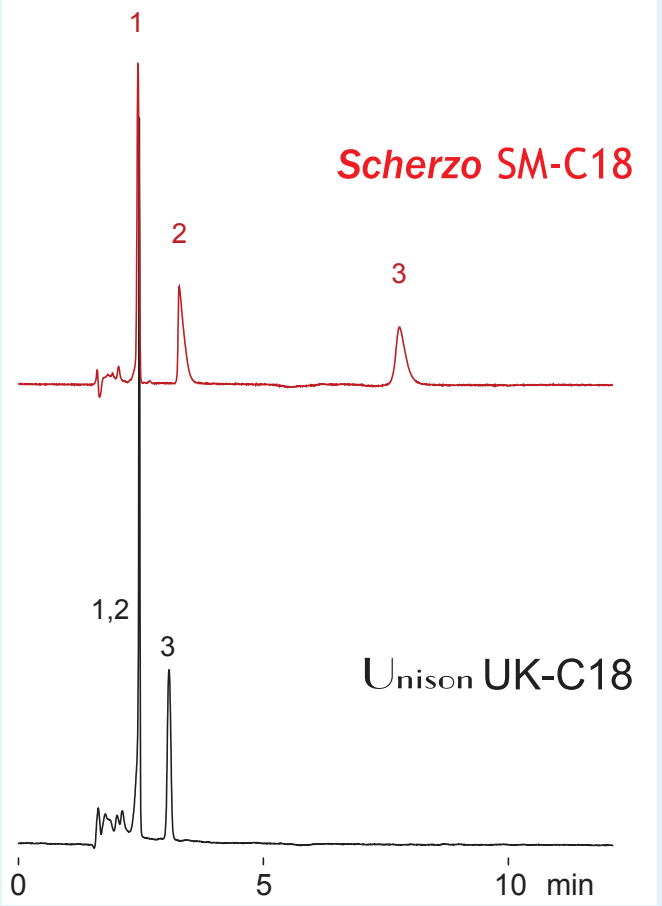


mevalonic acid

150 x 3 mm
5mM ammonium formate /acetonitrile = 90 /10
0.4 mL/min (10MPa), 37 deg.C
ELSD (spray chamber 20 deg.C,
drift tube 45 deg.C)
1 uL (10ug)

519

22 Acetic acid and trihaloacetic acids



1 CH_3COOH
acetic acid (100ppm)

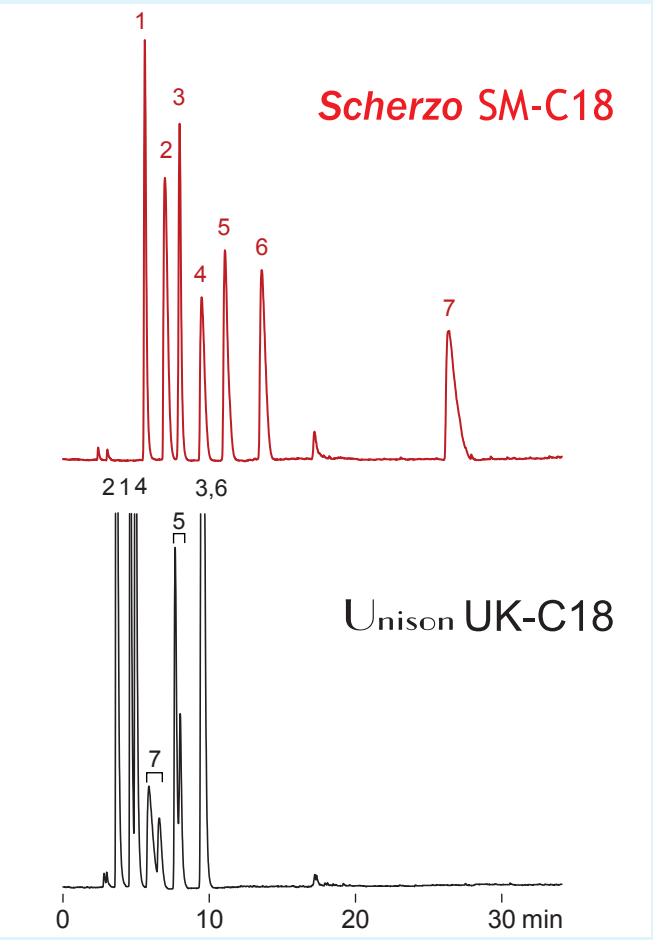
2 CF_3COOH
trifluoroacetic acid (100ppm)

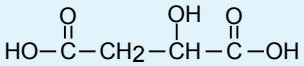
3 CCl_3COOH
trichloroacetic acid (9ppm)

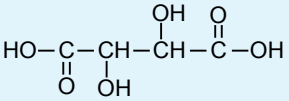
150 x 3 mm
100mM NH4H2PO4 / acetonitrile = 80 / 20
0.4 mL/min (9-10MPa), 37 deg.C, 210 nm
20 uL

523

23 Organic Acids

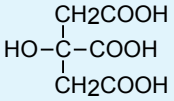


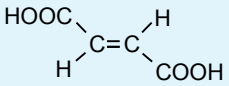
1 
malic acid

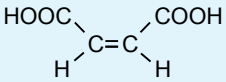
2 
tartaric acid

3 $HOOC(CH_2)_2COOH$
succinic acid

4 $HOOCCH_2COOH$
malonic acid

5 
citric acid

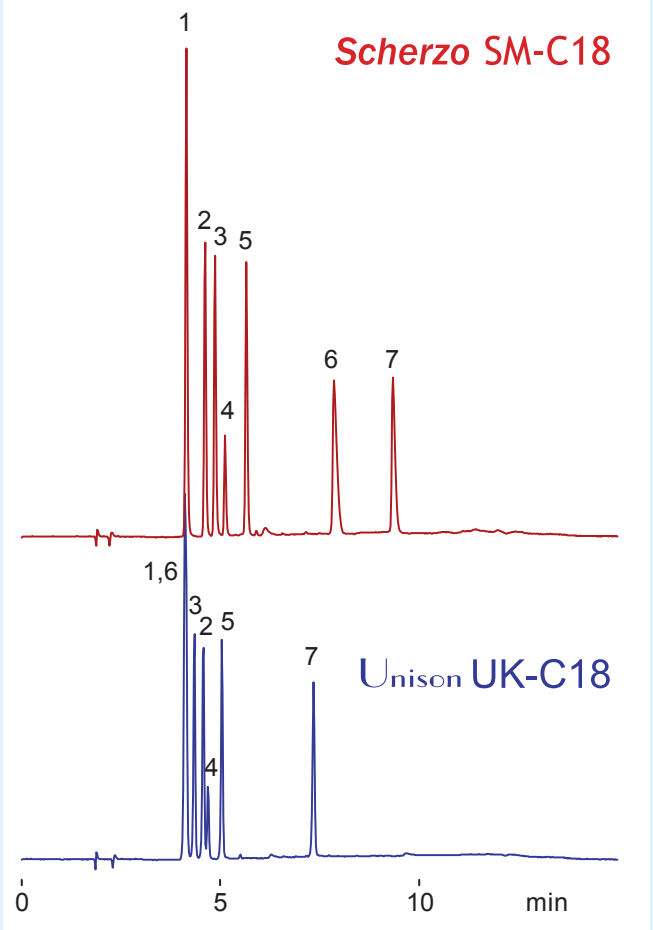
6 
fumaric acid

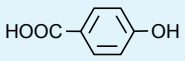
7 
maleic acid

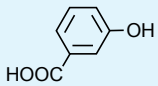
250 x 3 mm
A: water / formic acid = 100 /0.3
B: acetonitrile / formic acid = 100 /2
0-5 %B (0-13min), 50 %B (13-30min)
0.4 mL/min (13MPa), 37 deg.C
ELSD (spray chamber 19deg.C,
drift tube 43deg.C),
4 uL (3.8-6.2 ug)

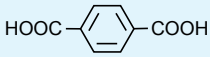
548

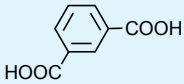
24 Aromatic Carboxylic Acids

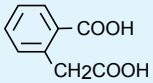


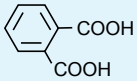
1 
p-hydroxybenzoic acid (pKa=4.5)

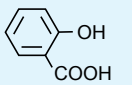
2 
m-hydroxybenzoic acid (pKa=4.3)

3 
terephthalic acid (p-) (pKa=3.5, 4.4)

4 
isophthalic acid (m-) (pKa=3.7, 4.6)

5 
homophthalic acid

6 
o-phthalic acid (pKa=2.7, 4.9)

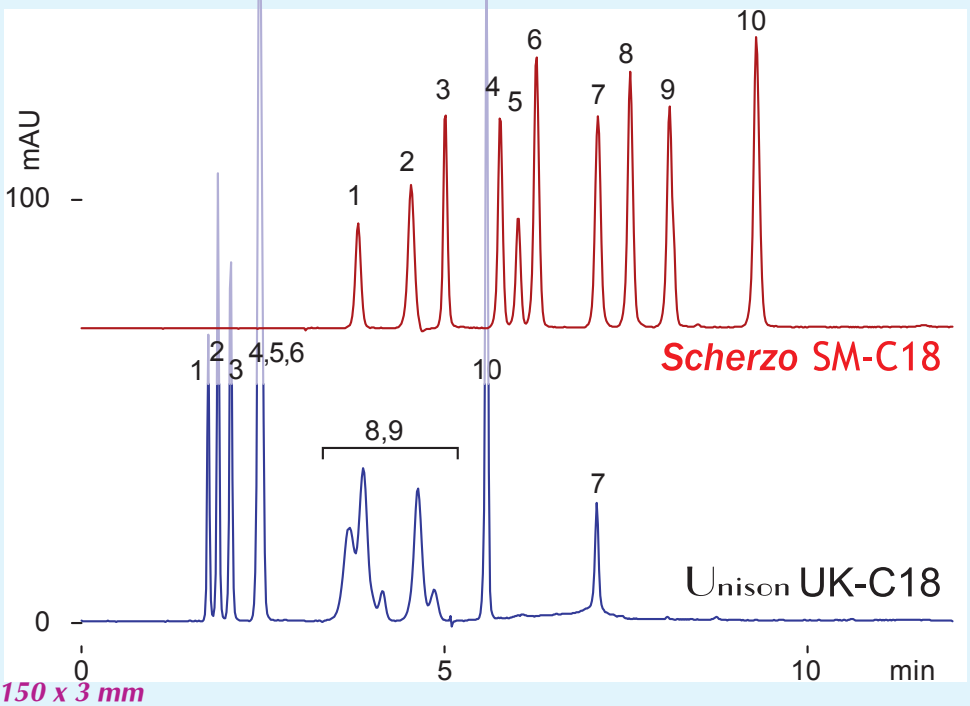
7 
o-hydroxybenzoic acid (pKa=2.9, 13.6)

150 x 3 mm
A: water / formic acid = 100 / 0.1
B: acetonitrile / formic acid = 100 / 2
20-70 %B (0-10min), 0.4 mL/min (9MPa)
37 deg.C, 275 nm, 2 uL (0.2-1 ug)

546

25 Mononucleotides

1 CMP
2 UMP
3 dCMP
4 dUMP
5 IMP
6 GMP

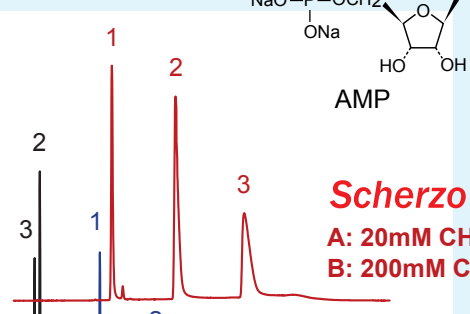


7 dTMP
8 AMP
9 dGMP
10 dAMP

150 x 3 mm
A: 5mM NH4H2PO4 : 5mM (NH4)2HPO4 = 1 : 1, pH 6.8
B: (10mM NH4H2PO4 : 10mM (NH4)2HPO4 = 1 : 1, pH 6.8) /acetonitrile = 50 /50
0-30 %B (0-10min), 0.4 mL/min (10MPa), 37 deg.C, 260 nm, 1 uL (0.2ug)

26 Adenosine phosphates (AMP, ADP, ATP)

1 AMP
2 ADP
3 ATP



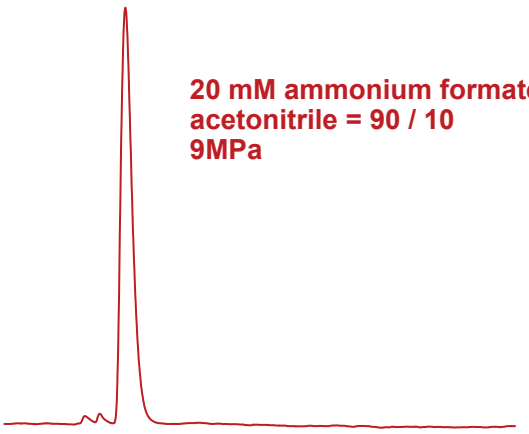
Scherzo SM-C18
A: 20mM CH_3COONH_4
B: 200mM CH_3COONH_4 /ACN = 70 /30

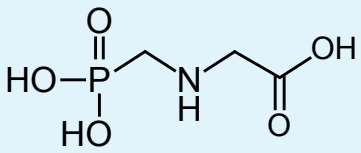
30 x 3 mm
0-100 %B (0-10min)
0.6 mL/min (5-6 MPa), 37deg.C, 260 nm, 0.8 uL (0.1-0.3ug)

Scherzo SM-C18
A: 5mM $NH_4H_2PO_4$: 5mM $(NH_4)_2HPO_4$ = 1:1, pH6.8
B: (20mM $NH_4H_2PO_4$: 20mM $(NH_4)_2HPO_4$ = 1:1, pH6.8) /ACN = 50 /50

Unison UK-C18
A: 5mM $NH_4H_2PO_4$: 5mM $(NH_4)_2HPO_4$ = 1:1, pH6.8
B: (20mM $NH_4H_2PO_4$: 20mM $(NH_4)_2HPO_4$ = 1:1, pH6.8) /ACN = 50 /50

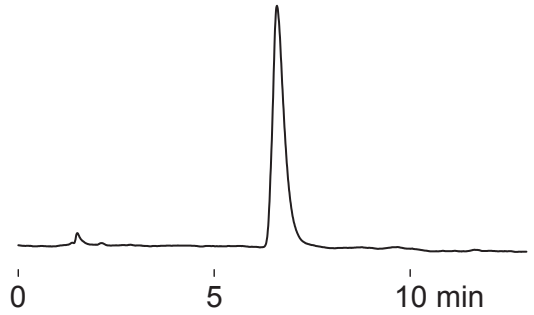
27 Glyphosate





glyphosate

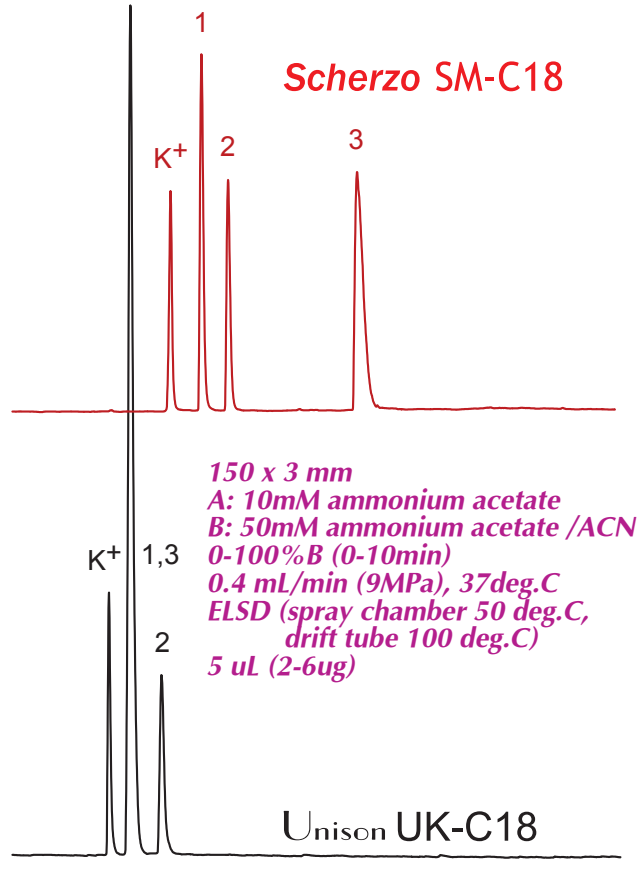
A: water / formic acid = 100 / 0.1
B: acetonitrile / formic acid = 100 / 0.1
0-50 %B (0-10min), 8MPa



Scherzo SM-C18, 150 x 3 mm
0.4 mL/min, 37 deg.C
ELSD (spray chamber 50 deg.C, drift tube 100 deg.C)
5 uL (5 ug)

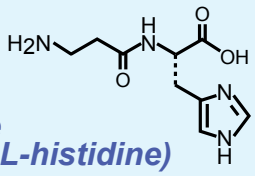
Courtesy of Takeo Sakuma, Ph.D
MDS Analytical Technologies (SCIEX), Canada

28 Anserine and Related Compounds



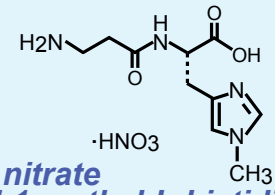
150 x 3 mm
A: 10mM ammonium acetate
B: 50mM ammonium acetate /ACN = 80 /20
0-100%B (0-10min)
0.4 mL/min (9MPa), 37deg.C
ELSD (spray chamber 50 deg.C,
drift tube 100 deg.C)
5 uL (2-6ug)

1



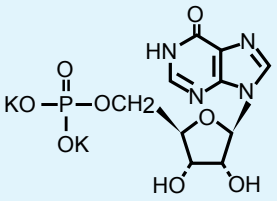
L- carnosine
(beta-alanyl-L-histidine)

2



L- anserine nitrate
(beta-alanyl-1-methyl-L-histidine nitrate)

3



Inosine 5'-monophosphate
dipotassium