### Base deactivation

NUCLEODUR® C18 Gravity and NUCLEODUR® C8 Gravity are based on the ultrapure NUCLEODUR® silica. Derivatization generates a homogeneous surface with a high density of bonded silanes (~ 18 % C for  $C_{18}$ , ~ 11 % C for  $C_8$ ). Thorough endcapping suppresses any unwanted polar interactions between the silica surface and the sample, which makes "Gravity" particularly suitable for the separation of basic and other ionizable analytes. Even strongly basic pharmaceuticals like amitriptyline are eluted without tailing under isocratic conditions. For a discussion of the different retention behavior of C<sub>18</sub> phases compared to C<sub>8</sub> phases see page 40.

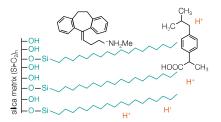
## Enhanced pH stability

One major disadvantage of silica stationary phases is limited stability at strongly acidic or basic pH. Cleavage of the siloxane bonding by hydrolysis, or dissolution of the silica will rapidly lead to a considerable loss in column performance. Conventional RP phases are usually not recommended to be run with mobile phases at pH > 8 or pH < 2 for extended periods of time. The special surface bonding technology and the low concentration of trace elements of NUCLEODUR® C18 and C8 Gravity allow for use at an expanded pH range from pH 1 to 11.

#### Benefits of enhanced pH stability

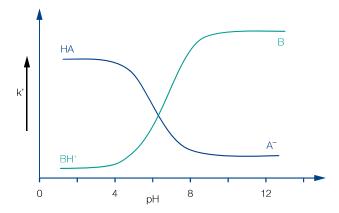
An expanded pH range is often required in method development. Many nitrogen containing compounds like basic drugs are protonated at acidic or neutral pH and exhibit poor retention on a standard C<sub>18</sub> phase. The retention behavior can be improved by working at a higher pH, where the analyte is no longer protonated, but formally neutrally charged, as a rule between pH 9-10. For acidic analytes it is exactly in inverse proportion, maximum retention can be attained at low pH.

#### Surface silanols at different pH values



The figure above shows the extent of protonation of surface silanols and of two exemplary analytes at acidic and alkaline pH. The following graph explains the general correlation between retention and pH.

## Correlation between retention and pH for basic and acidic compounds



An example how selectivity can be controlled by pH is the separation of the acid ketoprofen, the base lidocaine and benzamide. Under acidic conditions the protonated lidocaine is eluted very fast due to lack of sufficiently strong hydrophobic interactions

#### **Key features**

- Suitable for LC/MS and HPLC at pH extremes (pH 1-11)
- Superior base deactivation
- Ideal for method development

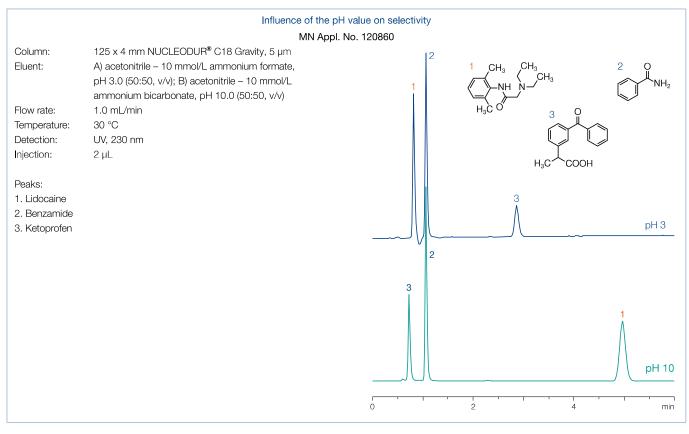
#### Technical data

- Octadecyl (C<sub>18</sub>) and octyl (C<sub>8</sub>) phase; multi-endcapped
- Pore size 110 Å; particle sizes 1.8  $\mu$ m, 3  $\mu$ m and 5  $\mu$ m for  $C_{18}$ , 1.8  $\mu m,$  3  $\mu m$  and 5  $\mu m$  for  $C_8;$  7  $\mu m,$  10  $\mu m,$ 12 µm and 16 µm particles for preparative purposes on request
- Carbon content 18% for C<sub>18</sub>, 11% for C<sub>8</sub>

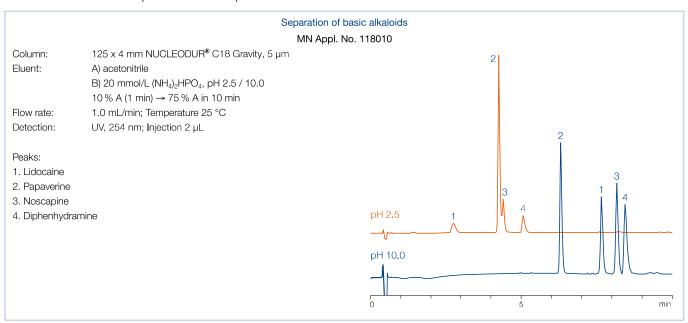
### Recommended applications

- USP listing L1
- Overall sophisticated analytical separations
- Compound classes separated include pharmaceuticals, e.g., analgesics, anti-inflammatory drugs, antidepressants; herbicides; phytopharmaceuticals; immunosuppressants

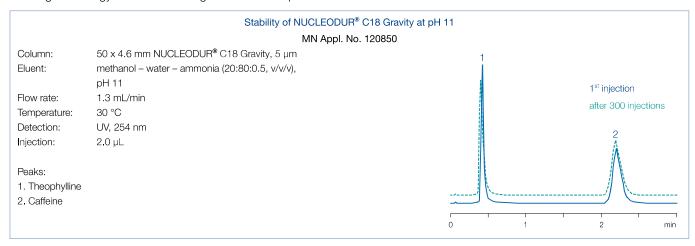
between analyte and  $C_{18}$  chains, while the formally neutral ketoprofen is eluted after about 3 min. However, at pH 10 a reversal of the elution order, with a visibly longer retention time for the basic lidocaine, is observed.



As mentioned above, pH stability of the stationary phase can be helpful for improving selectivity in method development. The following figure shows the separation of 4 basic drugs under acidic and basic conditions. At pH 2.5 the protonated analytes exhibit poor retention (early elution) and in addition an inadequate resolution for papaverine and noscapine, whilst the formally non ionized molecules can be baseline separated due to the better retention pattern at alkaline pH.



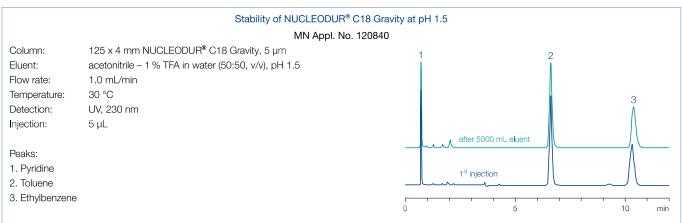
The following chromatogram demonstrates the stability of NUCLEODUR® C18 Gravity under alkaline conditions. The ultra-pure Gravity with its unique high density surface bonding technology withstands strong alkaline mobile phase conditions.



Even after 300 injections no loss of column efficiency - identified, e.g., by peak broadening or decrease in retention times - could be observed.

Under alkaline conditions dissolution of the silica support is possible, resulting in dead volume and thus peak broadening. It is worth mentioning, that this phenomenon also depends on type and concentration of buffers, as well as on the temperature. It is well known that the use of phosphate buffers, particularly at elevated temperatures, can reduce column lifetime even at moderate pH. If possible, phosphate buffers should be replaced by less harmful alternatives.

The following chromatograms show the excellent column stability of NUCLEODUR® C18 Gravity in acidic conditions. Retention times of all three compounds in the column performance test remain consistent and virtually unchanged, even after the column is run with 5000 mL eluent. Due to the extremely stable surface modification, no cleavage of the Si-O-Si bonding occurs, column deterioration is therefore successfully prevented.



## Ordering information

NUCLEODUR® C18 Gravity						
Analytical EC columns NUCLEODUR® C18 Gravity (pack of 1)						
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*		
250	4.6	5	760101.46	761903.30		
250	4	5	760101.40	761903.30		
250	3	5	760101.30	761903.30		
150	4.6	5	760103.46	761903.30		
250	3	3	760082.30	761902.30		
150	2	3	760083.20	761902.20		
125	4.6	3	760081.46	761902.30		
50	4.6	3	760080.46	761902.30		
100	4.6	1.8	760076.46	761901.30		
50	2	1.8	760079.20	761901.20		

\*Pack of 3, EC guard columns require column protection system REF 718966. For more information, see page 90.

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## Ordering information

NUCLEODUR® C8 Gravity						
Analytical EC columns NUCLEODUR® C8 Gravity (pack of 1)						
Length (mm)	ID (mm)	Particle size (µm)	REF	Guard columns*		
250	4.6	5	760753.46	761907.30		
250	4	5	760753.40	761907.30		
150	4.6	5	760752.46	761907.30		
150	4	5	760752.40	761907.30		
125	4.6	5	760751.46	761907.30		
125	4	5	760751.40	761907.30		
250	4.6	3	760659.46	761906.30		
50	3	3	760653.30	761906.30		
150	2	1.8	760759.20	761905.20		
50	3	1.8	760755.30	761905.30		

 $<sup>^{\</sup>star}\text{Pack of 3, EC guard columns require column protection system REF\,718966.} For more information, see page 90.$ 

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