Separation of Paracetamol and Related Substances following European Pharmacopoeia Method 9.4

Paracetamol (acetaminophen) is a common pain reliever and fever reducing drug taken individually, or in combination with other medications. A European Pharmacopeia (EP) method was developed for the analysis of paracetamol and its impurities, allowing manufacturers to monitor the purity of their paracetamol and the level of associated impurities.

Paracetamol contains fourteen impurities, all similar in structure. These structures are listed in Figure 1. Some of these impurities elute close to each other and can be difficult to separate. EP method 9.4 specifies a particle size of 2.7 µm and endcapped solid core octadecylsilyl silica gel in 2.1 x 100 mm. This corresponds to a HALO 90 Å C18, 2.7 µm column (Advanced Materials Technology). The method conditions for the separation are listed in Figure 2.

Figure 1: Structures of Paracetamol and 14 related substances

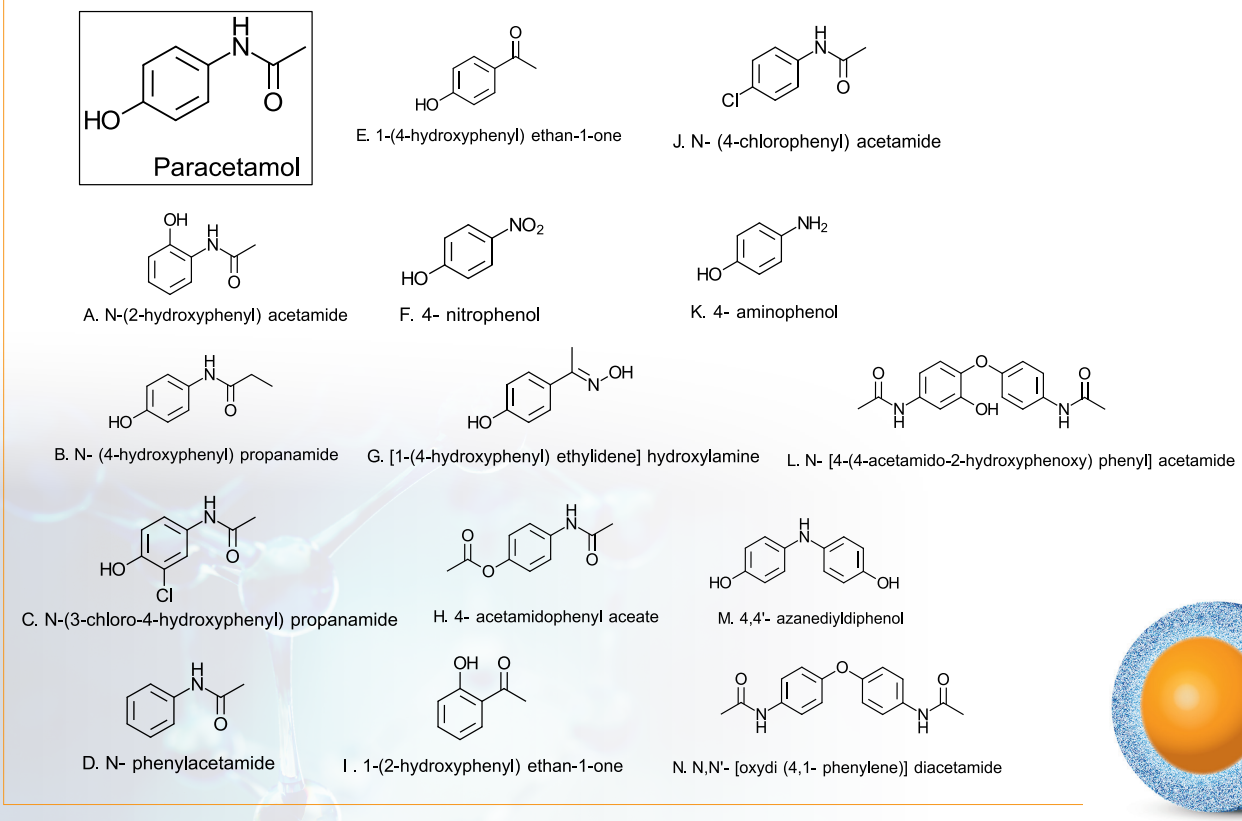


Figure 2: Method conditions for Paracetamol EP Method 9.4

METHOD CONDITIONS:

Mobile Phase A: pH 7 Phosphate buffer (prepared by dissolving 1.7g of potassium dihydrogen phosphate and 1.8g of dipotassium hydrogen phosphate in HPLC grade water and diluting to 1000mL with water
Mobile Phase B: Methanol

Gradient:	Time	%B
	0	5
	1	5
	10	10
	20	10
	40	34
	50	34

Instrument: Shimadzu Nexera X2
Wavelength: 254nm
Injection: 1 µl
Temperature: 30ºC
Flow Rate: 0.3 mL/min.

Paracetamol and 14 of its impurities are separated on a HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm column (pn: 92812-602) following the official European Pharmacopoeia 9.4 method. As indicated in the method, a HALO 90 Å C18 guard column (pn: 92812-102) is also used, which provides optimum protection for the HALO® HPLC column without sacrificing the column's efficiency. It is very important to use the appropriate guard column when running these tests since C18 bonded phases from different manufacturers can give different results. Guard columns from the same manufacturer as the analytical column are strongly recommended to avoid mismatches in selectivity. A comparison showing the results with and without the guard column is shown in Figure 3. The top chromatogram shows the results without the guard column while the bottom chromatogram shows the results with the guard column. The retention increases slightly with the use of the guard column. This increased retention also increases the resolution between critical pair impurities L and J from 1.61 to 2.87.

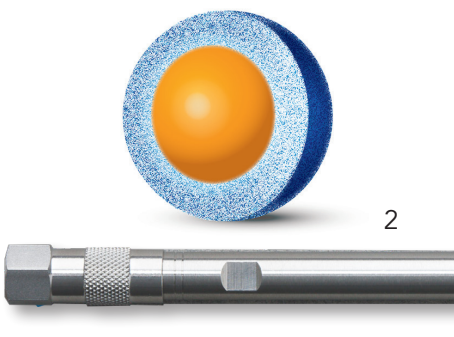
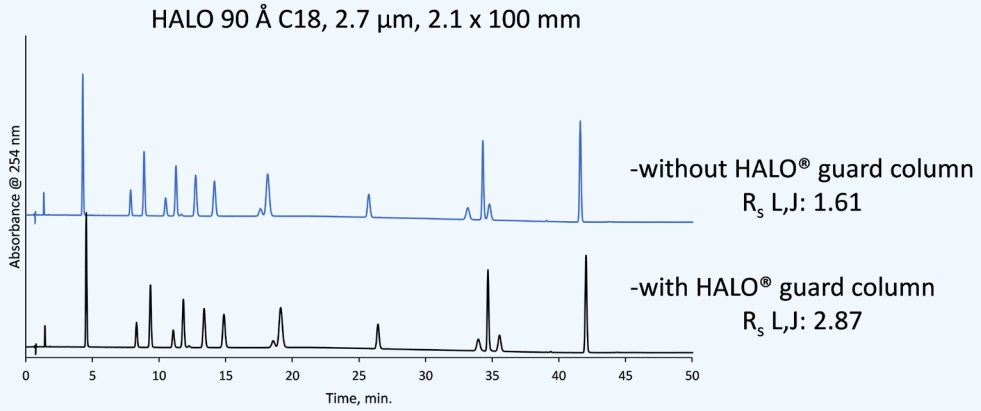
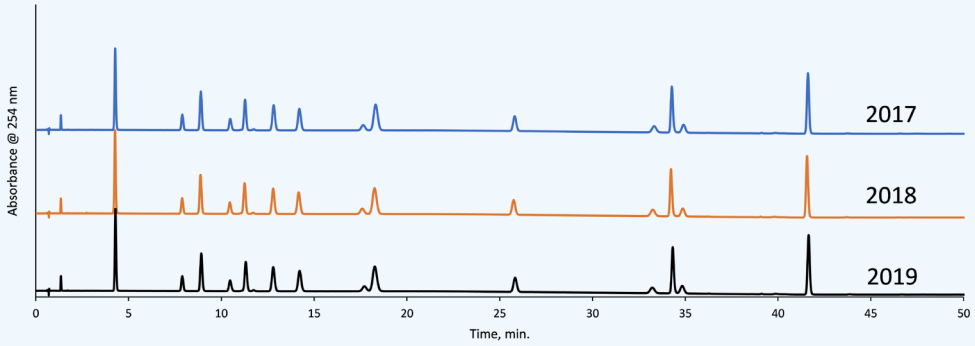


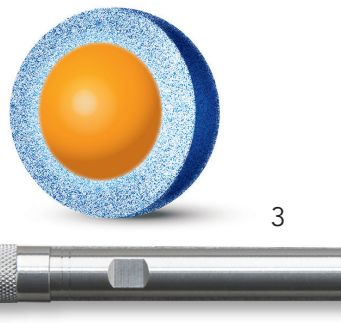
Figure 3: Separation of Paracetamol and 14 related substances with and without a HALO® guard column. Resolution is maintained between critical pair L and J.



AMT is one of few HPLC column manufacturers to produce its own silica. In fact, the entire column manufacturing process is completed in-house using 15 years of expertise and talents of AMT personnel. From the solid silica cores to the bonded Fused-Core® particles to the final loaded and QC tested column, customers can be confident that the HALO® products that they receive are reliable and reproducible. For example, the paracetamol separation shown in Figure 4 was run on three different lots spanning three years. Excellent reproducibility among the lots is observed.

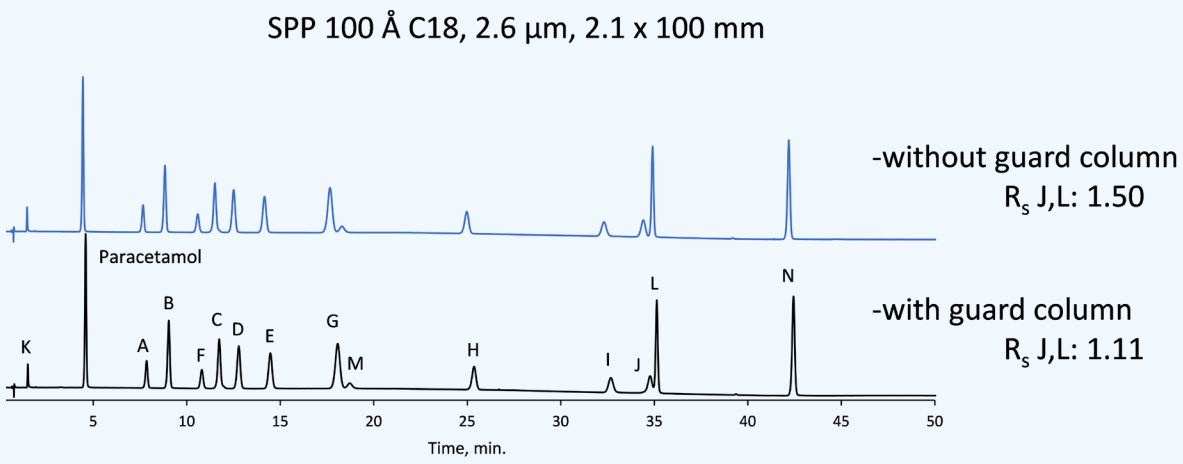
Figure 4: HALO® C18 columns spanning three years show excellent lot-to-lot reproducibility





For comparative purposes, the EP 9.4 method was performed using a competitor USP L1 designated SPP column, in this case a C18 2.6 µm, 100 Å, 2.1 x 100 mm column (with and without the appropriate guard column from the same manufacturer). See Figure 5. The top chromatogram shows the results without the guard column while the bottom chromatogram shows the results with the guard column. As was observed with the HALO® column, the presence of a guard column slightly increases the retention. However, in the case of the competitor column, the increased retention negatively impacts the resolution of impurities J and L since the resolution decreases from 1.50 to 1.11.

Figure 5: A competitor C18 SPP column is used to separate paracetamol and related compounds with and without the appropriate guard column.



Comparing the separation using the HALO® column to the separation using the other SPP column, a change in selectivity is observed between the critical pairs of M and G and L and J as is shown in Figure 6. Coelution of impurities J and L is also observed on the other SPP column. It is important to note that not all C18 columns will provide the same selectivity and peak elution order changes are possible. It cannot be assumed that C18 columns from different manufacturers will perform the same for the same analysis since the selectivity can be different as was demonstrated with this example.

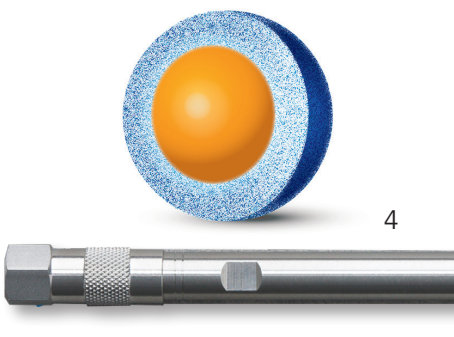
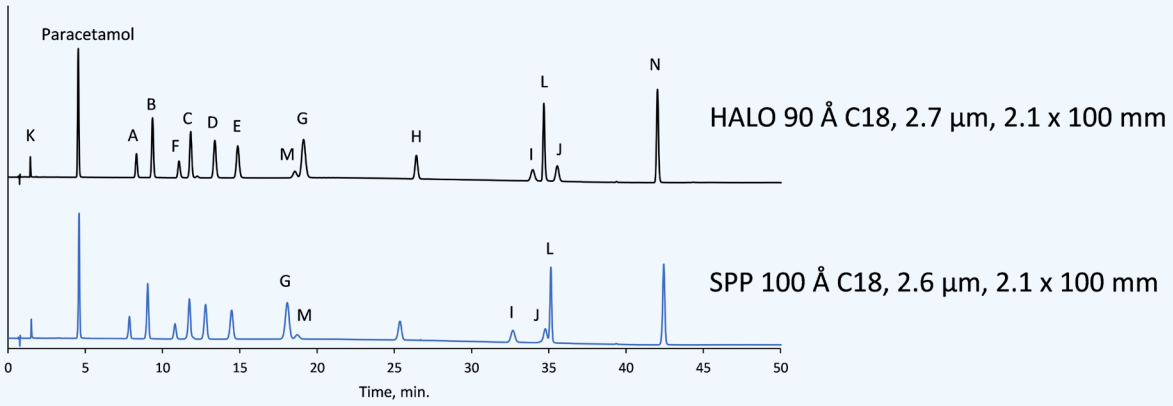


Figure 6: Paracetamol and 14 related substances separated using HALO C18 and another SPP C18 with appropriate guard columns from the corresponding manufacturer.

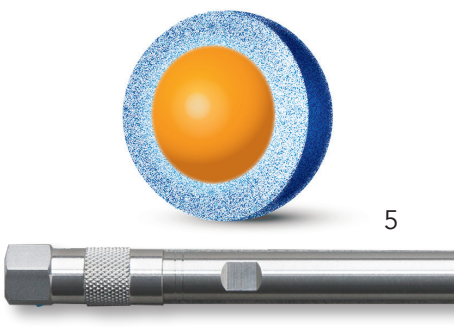


CONCLUSION

HALO® C18 2.7 µm columns are specified in EP 9.4 for paracetamol and its impurities. The column is an ideal choice for paracetamol and related substances separations due to its ability to resolve all of the critical pairs both with and without a guard column while other columns on the market have reduced resolution. When directly comparing the two columns (with appropriate guard columns) the resolution is much higher on the HALO® column. Finally, HALO® manufacturing provides stable and reproducible separations as shown by the multi-year lot-to-lot example.

REFERENCE

J Chromatogr A, 1223 (2012) 24-34 J.L Rafferty, J.I. Siepmann, and M.R. Schure.



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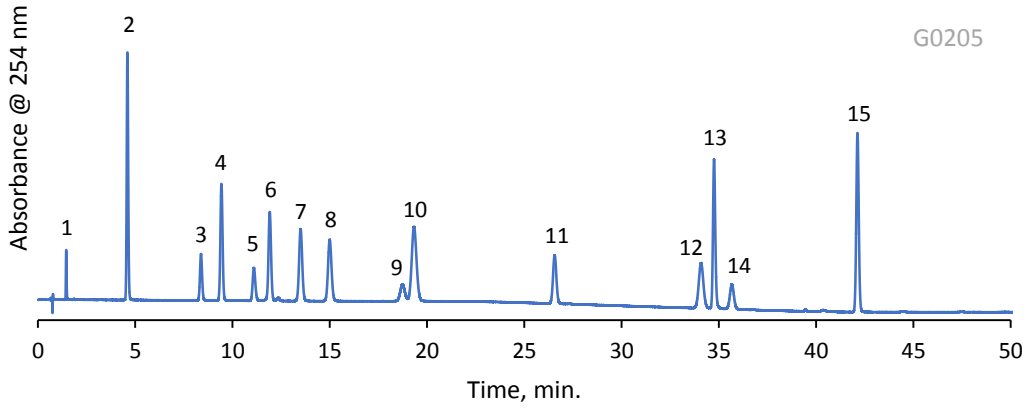
Application Note: 211-EP

Paracetamol Impurities: European Pharmacopoeia 9.4 Method



PEAK IDENTITIES:

1. Impurity K
2. Paracetamol
3. Impurity A
4. Impurity B
5. Impurity F
6. Impurity C
7. Impurity D
8. Impurity E
9. Impurity M
10. Impurity G
11. Impurity H
12. Impurity I
13. Impurity L
14. Impurity J
15. Impurity N

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100mm
Part Number: 92812-602
Guard Column: HALO 90 Å C18, 2.7 µm, 2.1 x 5mm
Part Number: 92812-102
Guard Column Holder: Part Number: 94900-001
Mobile Phase A: Phosphate Buffer (1.7g. potassium dihydrogen phosphate and 1.8g. dipotassium hydrogen in 1000mL)
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	5
1.0	5
10.0	10
20.0	10
40.0	34
50.0	34

Flow Rate: 0.3 mL/min
Initial Pressure: 246 bar
Temperature: 30°C
Detection: 254 nm, PDA
Injection Volume: 1 µL
Sample Solvent: 85/15 Water/ MeOH
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Paracetamol (acetaminophen) is a common pain relief and fever medication taken individually, or in combination with other medications. An analysis of paracetamol and 14 of its impurities are separated on a HALO 90Å C18 column following the official European Pharmacopoeia 9.4 method. Baseline resolution is obtained for all compounds including critical pairs of impurity M/G and impurities I/L/J. A HALO 90Å C18 guard column is also used in order to provide optimum protection for your HALO® HPLC column without sacrificing the column's efficiency.

-For more information visit the *Under the HALO*

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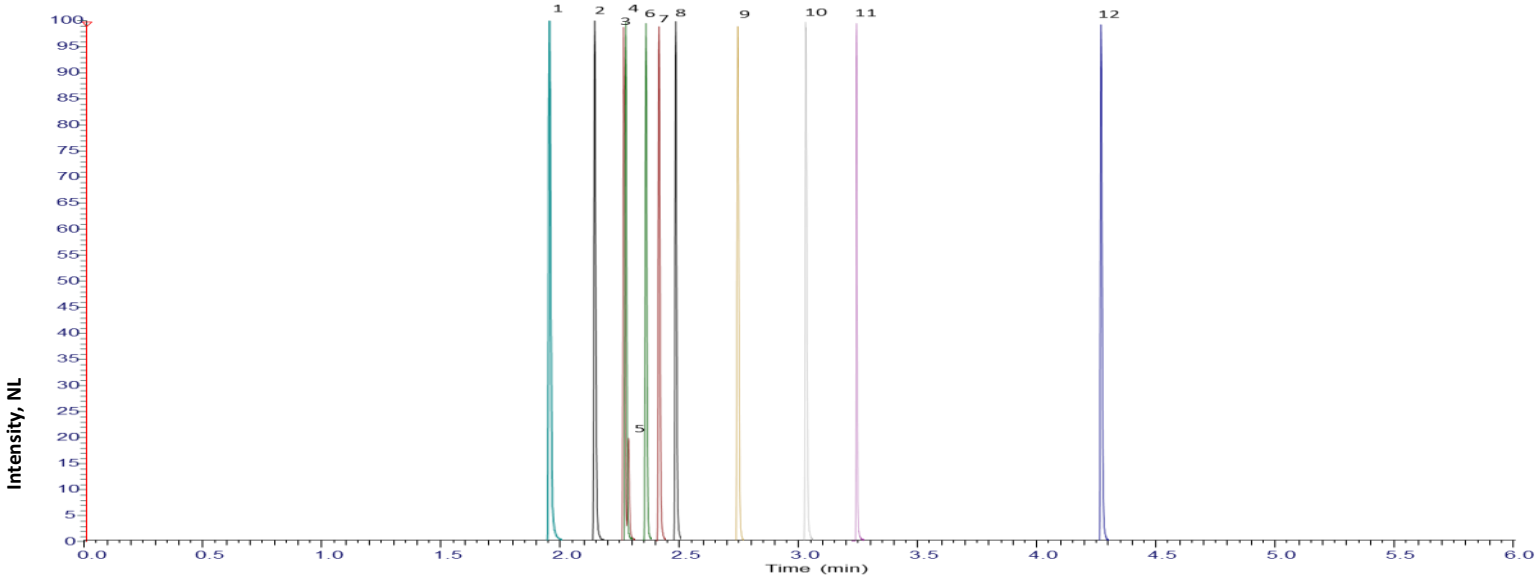
Application Note: 205-TOX

LC-MS Separation SAMHSA 5 Panel on HALO® Biphenyl 2 µm

G0199



TEST CONDITIONS:

Column: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100
Part Number: 91812-611
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: Methanol/0.1% Formic acid
Gradient:

Time	%B
0.0	5
4.00	98
5.00	98
5.01	5
7.00	END

Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Injection Volume: 2 µL
Sample Solvent: 95/5 MeOH/Water
LC System: Shimadzu Nexera X2

MS CONDITIONS:

Detection:: +ESI MS
Mass Spectrometer: Thermo Exactive HF
Sheath gas flow rate: 50 (arbitrary units)
Aux gas flow rate: 13 (arbitrary units)
Sweep gas flow rate: 0 (arbitrary units)
Spray voltage: 3.50 k V
Cap temp: 263 °C
S-lens RF level: 70 V
Aux gas heater temperature: 425 °C

PEAK IDENTITIES:

1. Morphine (MH^+= 286.341 g/mol)
2. Amphetamine (MH^+= 136.206 g/mol)
3. Methamphetamine (MH^+= 150.237 g/mol)
4. MDA (MH^+= 180.221 g/mol)
5. Phentermine (MH^+= 150.233 g/mol)
6. Codeine (MH^+= 300.364 g/mol)
7. 6-MAM (MH^+= 328.380 g/mol)
8. MDMA (MH^+= 194.246 g/mol)
9. MDEA (MH^+= 208.271 g/mol)
10. Benzoylecgonine (MH^+= 290.331 g/mol)
11. PCP (MH^+= 244.387 g/mol)
12. THC-COOH (MH^+= 345.415 g/mol)

The 2 µm HALO Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between methamphetamine and phentermine, (peaks 3 and 5, respectively). The SAMHSA 5 panel consists of amphetamines, cocaine, marijuana, opiates, and phencyclidine (PCP).

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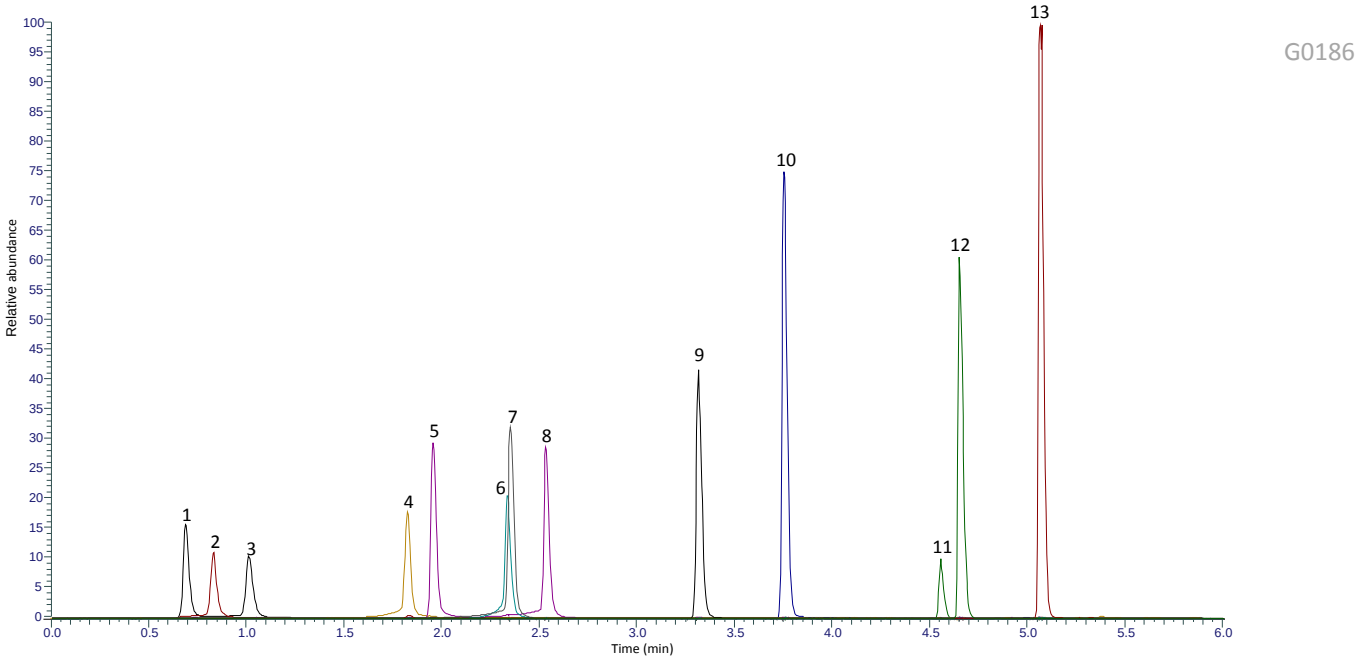
Application Note: 192-OP

LC-MS Separation of Pain Management Opiates on HALO® 2 µm Biphenyl



TEST CONDITIONS:

Columns: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100mm
Part Number: 91812-611
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: Acetonitrile/0.1% Formic acid
Gradient:

Time	%B
0.0	10
2.22	20
5.00	60
5.50	60
5.51	10
6.50	END

Flow Rate: 0.4 mL/min
Initial Pressure: 325 bar
Temperature: 40 °C
Injection Volume: 1 µL
Sample Solvent: 95/5 water/acetonitrile
LC System: Shimadzu Nexera X2
Detection: +ESI MS

PEAK IDENTITIES:

1. Morphine (m/z 286)
2. Oxymorphone (m/z 302)
3. Hydromorphone (m/z 286)
4. Naloxone (m/z 328)
5. Codeine (m/z 300)
6. Naltrexone (m/z 342)
7. Oxycodone (m/z 316)
8. Hydrocodone (m/z 300)
9. cis-Tramadol (m/z 264)
10. Meperidine (m/z 248)
11. Fentanyl (m/z 337)
12. Buprenorphine (m/z 468)
13. (+/-) - Methadone (m/z 310)

The 2 µm HALO Biphenyl is an ideal choice for high throughput analysis of drug panels, in which isobaric species separation is needed. Note the resolution between codeine and hydrocodone, (peaks 1 and 3, respectively) and morphine and hydromorphone (peaks 5 and 8, respectively).

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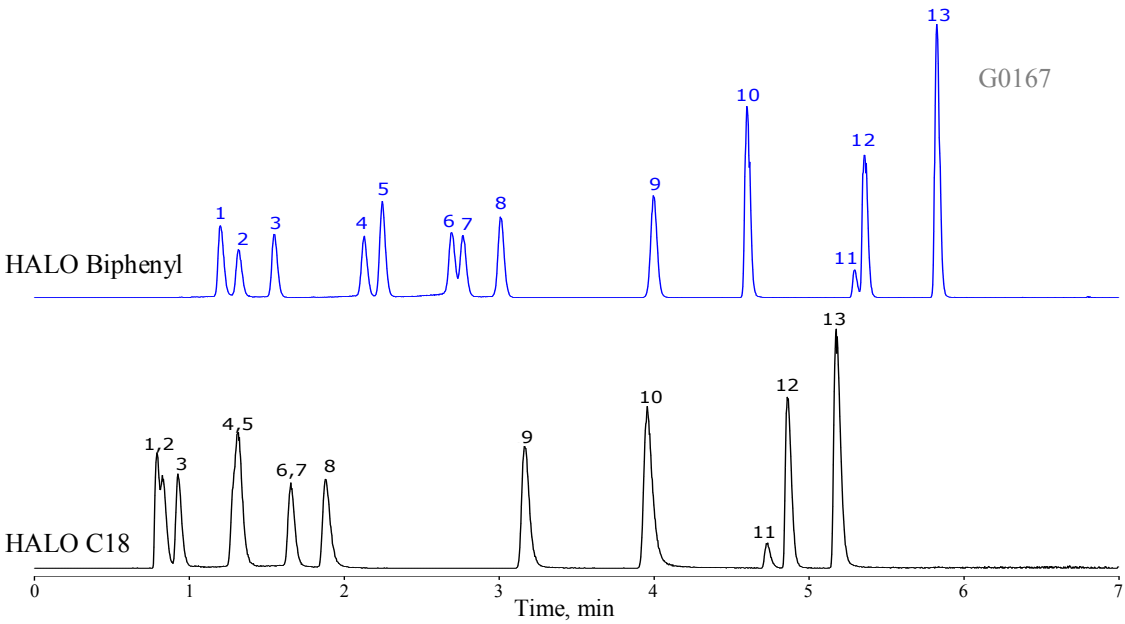
Application Note: 173-OP

Pain Management Panel Comparison on HALO Biphenyl and C18



PEAK IDENTITIES:

1. Morphine
2. Oxymorphone
3. Hydromorphone
4. Naloxone
5. Codeine
6. Naltrexone
7. Oxycodone
8. Hydrocodone
9. cis-Tramadol HCl
10. Meperidine
11. Fentanyl
12. Buprenorphine
13. (±)-Methadone

TEST CONDITIONS:

Columns: HALO 90Å Biphenyl, 2.7 µm, 2.1 x 100mm
Part Number: 92812-611
HALO 90Å C18, 2.7 µm, 2.1 x 100mm
Part Number: 92812-602
Mobile Phase A: water/0.1% formic acid
Mobile Phase B: ACN/0.1% formic acid
Gradient: 0–3 min. 10–20% B
3–3.5 min. 20–100% B
3.5–6 min. hold at 100% B
Flow Rate: 0.3 mL/min
Temperature: 30°C
Instrument: Agilent 1290
Dwell Volume: 0.19 mL
Injection Volume: 2 µL
Sample Solvent: water: methanol, 99:1

MS System: Agilent 6210 TOF
ESI: +4 kV
Gas Temperature: 360 ˚C
Gas Flow: 12 L/min
Nebulizer: 50 psi
Scan Rate: 5 spectra/s
Fragmentor: 175 V
Skimmer: 65 V
Octopole RF: 250 V

The HALO Biphenyl phase provides greater retention and improved resolution for the polar analytes in this mixture of pain management drugs. Compound pairs 1/2 and 4/5 are baseline separated using the HALO Biphenyl column, but co-elute on the HALO C18 column. Analytes 6 and 7 are partially resolved on the HALO Biphenyl column, but they co-elute using the HALO C18 column. These bonded-phase selectivity differences are very useful for method development, and provide a basis for LC-MS analyses of large pain medicine panels.

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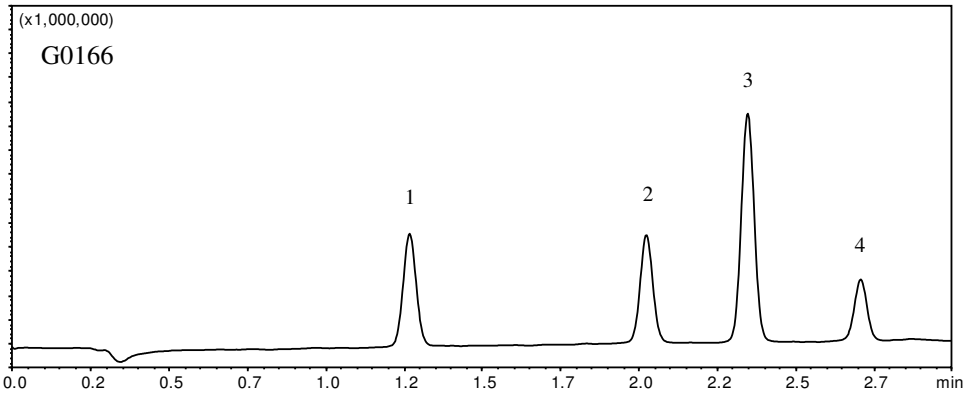
Application Note: 172-OP

LC-MS Separation of Fentanyl and Analogues in Synthetic Urine



Time, min.

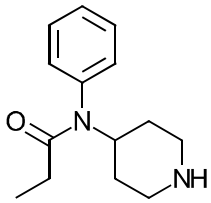
PEAK IDENTITIES:

1. Norfentanyl TIC/ 233
2. Acetyl Fentanyl TIC/ 323
3. Fentanyl TIC/ 337
4. Sufentanil TIC/ 387

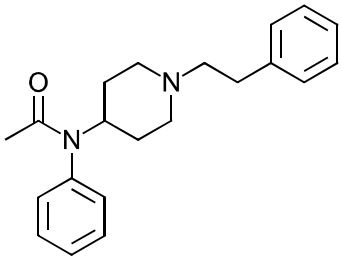
TEST CONDITIONS:

Column: HALO 90Å Biphenyl, 2.7 µm, 2.1 x 50mm
Part Number: 92812-411
Mobile Phase A: Water/ 0.1% Formic acid/ 10mM Ammonium formate
Mobile Phase B: Methanol/ 0.1% Formic acid/ 10mM Ammonium formate
Gradient: 40-90% B in 3 minutes
Flow Rate: 0.8 mL/min
Initial Pressure: 380 bar
Temperature: 30°C
Injection Volume: 0.5 µL
Sample Solvent: Surine Negative Urine
LC System: Shimadzu Nexera
MS: Shimadzu LCMS 2020 (single quadrupole
ESI: 4.5 kV
Heat Block: 300˚C
Nebulizing Gas Flow: 1.3 L/min

STRUCTURES:

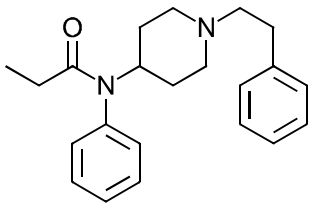


Norfentanyl

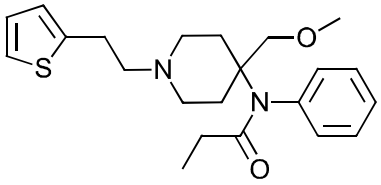


Acetyl Fentanyl

A mixture of fentanyl and some of its analogues spiked into synthetic urine are separated on a HALO Biphenyl column using LC-MS detection. These opioids are known to be much more potent than heroin and have become a significant contributor towards the opiate crisis in America.



Fentanyl



Sufentanil

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