Dimensions	Catalog Number
4.6 x 50 mm	803-0505C
4.6 x 150 mm	803-0510C
10 x 50 mm	803-1005C
10 x 100 mm	803-1010C
20 x 50 mm	803-2005C
20 x 100 mm	803-2010C
20 x 250 mm	803-2025C

WHAT'S IN FLUOROFLASH® HPLC COLUMNS? FluoroFlash® HPLC columns are packed with fluorous silica gel, which is silica gel containing a perfluorooctylethylsilyl ($Si(CH_2)_2C_8F_{17}$) bonded phase. FluoroFlash® fluorous silica gel separates compounds primarily based on fluorine content while compound polarity and other factors play lesser roles. This makes it the material of choice for both analytical and preparative separations of organofluorine compounds.

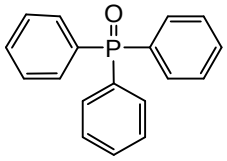
IS MY FLUOROFLASH® COLUMN LIKE A REVERSE PHASE (RP) COLUMN? Yes and no. In practical aspects of use, FluoroFlash® columns resemble standard reverse phase (C18 RP) columns. In other words, if you are familiar with reverse phase chromatography, then you already know how to use a FluoroFlash® column. What's very different is the separation, especially with fluorinated molecules. The following table provides a comparison of reverse phase and fluorous silica gels.

Features of Fluoro*Flash*® HPLC Columns at a Glance

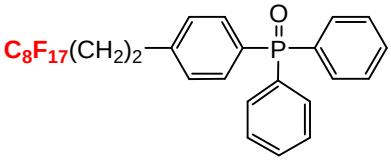
Feature	Similar To RP	Different From RP	Comments
absolute and relative R_f's		√√	fluorinated compounds are retained by fluorine content
Separation mechanism		√√	fluorous silica is hydrophobic, organophobic and fluorophilic
organic eluting solvents	√		alcohols, acetonitrile, etc.
water content in eluent		√	use sparingly; a little water goes a long way
sample pretreatment	√		filter to avoid particulates
injection solvents	√		use organic mobile phase, DMF, THF, etc.
loading levels	√		higher loadings can be used for fluorous/non-fluorous separations
storage and handling	√		store under MeOH
cleaning	√		clean with MeOH or CH_3CN, use 1-10% THF if needed
buffers and additives	√		0.1% TFA, other additives are commonly used
back pressure	√		lower water content helps keep back pressure down
lifetime	√		columns are reusable and durable
flow rates	√		vary with column size/length
preparative use	√		remove baseline impurities by spe with fluorous or regular silica
detectors	√		UV, light scattering, MS, etc., all compatible

HOW CAN I GET STARTED WITH MY NEW COLUMN? It's fast and easy. Just use as you would a standard reverse phase column. Columns are factory-tested and come packed in methanol. A test injection with the "three phosphine oxide" test mixture was run on the column prior to shipment and that chromatogram is included. This mixture contains non-fluorous triphenylphosphine oxide ($O=PPh_3$), light fluorous $C_8F_{17}(CH_2)_2C_6H_4P(=O)Ph_2$ and medium fluorous $[C_6F_{13}(CH_2)_2C_6H_4]_2P(=O)Ph_2$. These are stable compounds that provide a calibration over a wide range of conditions.

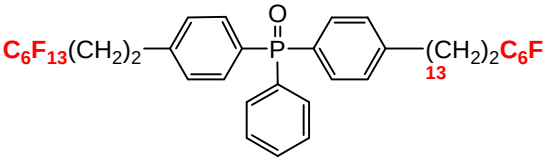
The "three" phosphine oxide" test mixture



triphenylphosphine oxide;
a non-fluorous control;
not retained unless very
high water content is used

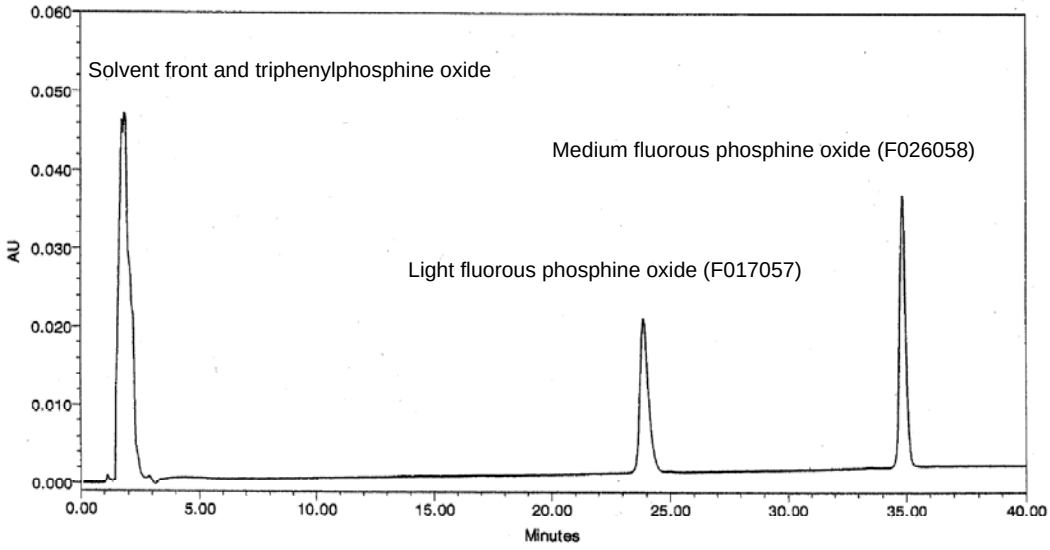


a light fluorous phosphine oxide;
F017057
typical of FTI's light reagents
and catalysts for F-SPE separation



a medium fluorous phosphine oxide;
F026058
strongly retained on HPLC
can also be removed by liq-liq extraction

Typical HPLC Chromatogram of the "Three Phosphine Oxide" Standard Mixture



Conditions: 85% MeOH/H_2O – 100% MeOH, 0–30 min, then 100% MeOH
4.6 x 150 mm Fluoro*Flash*® column (803-0510C), 1 mL/min

WHAT SOLVENTS DO YOU RECOMMEND AS MOBILE PHASES? Common solvents in rough order of fluorophilicity (ability to elute fluorous compounds) are isocratic mixtures or gradients of methanol/water, acetonitrile/water or DMF/water. A good starting point is a fast gradient of 60/40 methanol/water up to 100% methanol. Or use acetonitrile in place of methanol if you prefer. You can fine tune from there once you see the retention times of your compounds. What if nothing comes off? It's not very likely with fluorous compounds in our experience, but if you have very polar (especially charged) or lightly fluorinated compounds, you might need more water. At the other extreme, if you have loads of fluorines, you might need to bring in THF or another fluorophilic solvent. Do that sparingly, since THF is a powerful eluent.

WHAT'S THE DEAL WITH WATER? Water is the ultimate fluorophophic (fluorous-hating) solvent and it is typically an important component of the mobile phase. A good rule of thumb is that a little water goes a long way in retaining fluorous compounds. For example, the retention time of the second phosphine oxide in the test mixture changes from about 5 minutes in 100% MeOH to more than 20 minutes in 90/10 methanol/water, while the retention time of the third phosphine oxide changes from about 9 minutes to almost two hours [4.6 x 150 mm column, 1 mL/min flow rate]. Triphenylphosphine oxide, in contrast, continues to elute at the solvent front even with 80/20 methanol water. Gradient elutions are generally preferred since large separations often make run times under isocratic conditions too long.

WHAT CAN I DO WITH MY FLUOROUS HPLC COLUMN? Many things. Here a few highlights:[1]

- analyze and purify fluorous compounds[1]
- separate and quantitate fluorous analytes in proteomics and other areas[2]
- develop/predict conditions for fluorous solid phase extractions[1]
- purify fluorous-tagged oligopeptides and oligonucleotides after solution phase or solid phase synthesis[3]
- analyze organofluorine compounds (use high water content if you have only one or a few fluorines)
- demix tagged components if fluorous quasiracemic synthesis or fluorous mixture synthesis[4]

CAN I SEPARATE COMPOUNDS WITHOUT FLUORINE ATOMS ON MY COLUMN? Sure, give it a try because fluorous columns are quite different for other reverse phase and normal phase columns. But remember that the columns are organophobic. Compared to regular reverse phase columns, you will need to use a much higher water content in the mobile phase for non-fluorinated compounds.

HOW TO SEPARATE BASIC COMPOUNDS WITH THE FLUOROUS COLUMN? Fluorous and non-fluorous compounds with free amino groups could have longer retention time and bad peak shape on the fluorous column. We have found that using <0.1% TFA buffered MeOH-H_2O mobile phase (pH around 2-3), the basic compounds can be well separated and have good peak shape. The basic buffer with 0.05% NH_4OH (pH around 9) can also be used for the separation of basic compounds. For the consideration of life time of the fluorous column, we suggest to bring to the mobile pH back to normal after using the buffer.

REFERENCES:

1) Curran, D. P. Separations with Fluorous silica gel and related materials. *The Handbook of Fluorous Chemistry*; Wiley-VCH: Weinheim, 2004; pp 101-127. Curran, D. P. Fluorous reverse phase silica gel. A new tool for preparative separations in synthetic organic and organofluorine chemistry. *Synlett* 2001, 1488-1496.
2) Brittain, S. M.; Ficarro, S. B.; Broack, S.; Peters, E. C. Enrichment and analysis of peptide subsets using fluorous affinity tags and mass spectrometry. *Nature Biotechnology* 2005*, 23*, 463-468.
3) de Visser, P. C.; van Helden, M.; Filippov, D. V.; van der Marel, G. A.; Drijfhout, J. W.; van Boom, J. H.; Noort, D.; Overkleeft, H. S. A novel, base-labile fluorous amine protecting group: synthesis and use as a tag in the purification of synthetic peptides. *Tetrahedron Lett.* 2003*, 44*, 9013-9016.
4) Zhang, W.; Luo, Z.; Chen, C. H. T.; Curran, D. P. Solution-phase preparation of a 560-compound library of individual pure mappicine analogues by fluorous mixture synthesis. *J. Am. Chem. Soc.* 2002*, 124*, 10443-10450.