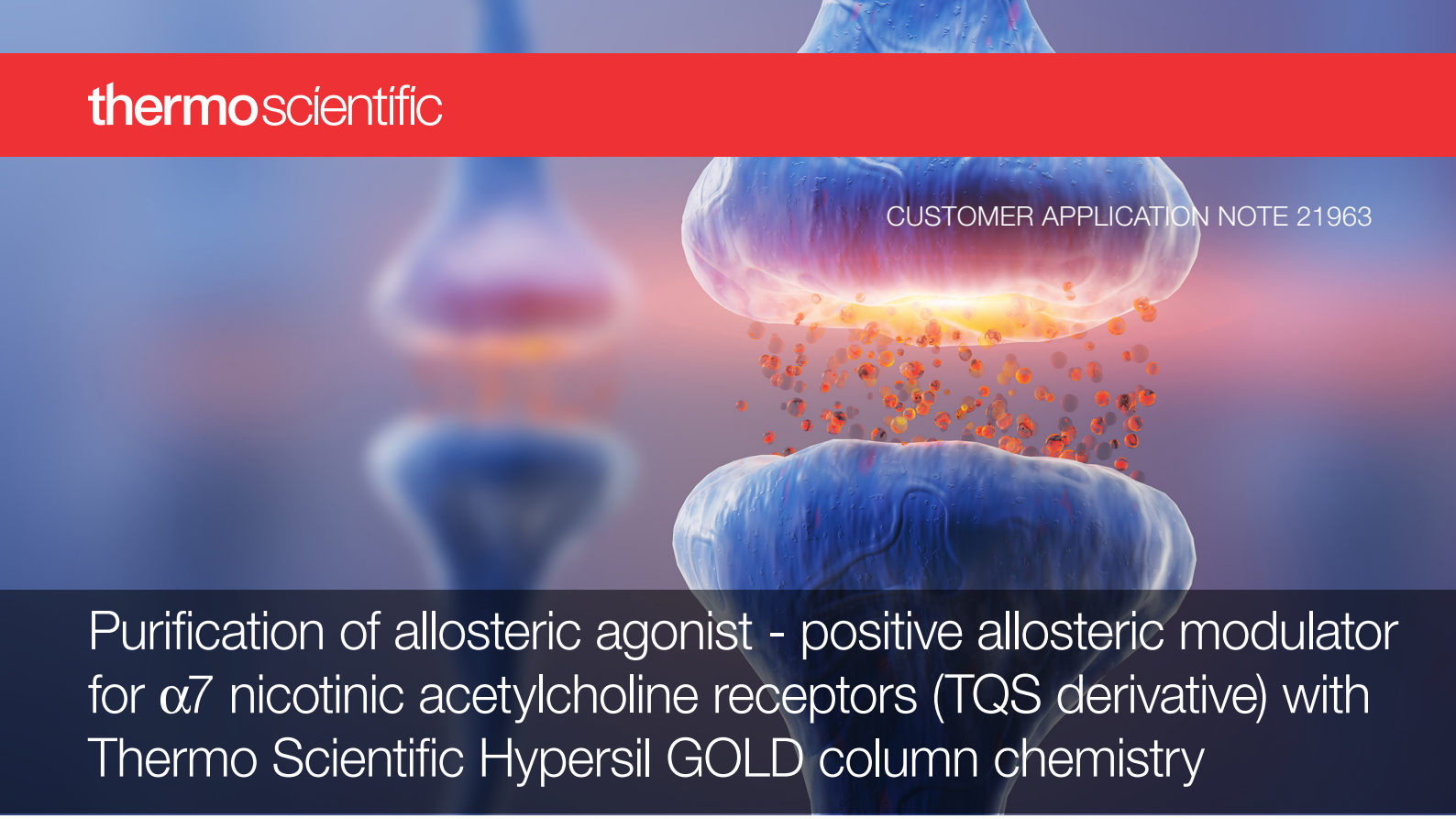
thermo scientific

CUSTOMER APPLICATION NOTE 21963



Purification of allosteric agonist - positive allosteric modulator for α7 nicotinic acetylcholine receptors (TQS derivative) with Thermo Scientific Hypersil GOLD column chemistry

Commentary and practical tips for mastering compound purification easily and effectively

Authors

Lætitia Bailly,[1] Emilie Petit,[1] Mike Baynham,[2] and Rainer Bauder[2]

[1]COBRA, INSA de Rouen, IRCOF, France

[2]Thermo Fisher Scientific, Runcorn, UK

Keywords

Preparative HPLC, analytical HPLC, pilot run, Chromeleon 7.2 software, Hypersil GOLD UHPLC, HPLC and preparative LC columns, generic guidance for novice and expert, research, synthesis and purification of positive allosteric modulators, Alzheimer's Disease, 4-(naphthalen-1-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide, TQS, organophosphates, poisoning, reactivation

Goal

Showcase a structured approach on how to achieve successful purification of synthetic, α7 nAChR, biological active compound for research and discovery. Presentation of an effective strategy and tools to accomplish high substance purity with minimized target substance loss.

Introduction

The general objective of COBRA (Chimie Organique Bioorganique Réactivité et Analyse, or translated - Organic and Bioorganic Chemistry, Reactions and Analysis) is to develop new innovative synthesis methods and apply them to the various sub-domains of the associated disciplines. The sub domains are bioorganic chemistry, pharmaceutical chemistry, environmentally sustainable chemistry, and material science related chemistry.

The bioorganic chemistry team from COBRA works on novel antidotes. Derived from research on Alzheimer's disease[1] where TQS, an α7 nicotinic acetylcholine receptor agonist, was found to be able to switch on deactivated receptors, the laboratory uses derivatives of TQS to pursue similar reactivation for individuals exposed to organophosphorus pesticides or warfare agents.

ThermoFisher SCIENTIFIC

Organophosphorus pesticides are a serious public health issue worldwide with over 200,000 fatalities annually. Organophosphorus warfare agents present a persistent threat to the general population because of armed conflicts (e.g., Gulf War) and terrorist attacks (e.g., subway attacks in Japan in 1995). These compounds irreversibly inhibit acetylcholinesterase (AChE, EC 3.1.1.7), which plays an essential role in neurotransmission. Over the last 60 years, pyridinium oxime compounds have been widely used as antidotes to treat these intoxications.[2] Despite decades of research in this field, there is no efficient and general reactivator for organophosphorus-inhibited AChE. Interest in this field has increased since the September 2001 terrorist attacks in the U.S.A. The important and recent advances in research on organophosphorus-inhibited AChE is significant.

Experimental

Instrumentation

The purification of synthesized TQS was achieved by using a Thermo Scientific™ UltiMate™ 3000 system and an Interchim puriFlash® 4250 Preparative LC system.

The analytical UltiMate 3000 system consists of:

- LPG-3400 RS pump module (P/N 82122708)
- SR-3000 solvent rack (P/N 50559200)
- WPS-3000TRS autosampler (P/N 8212791)
- TCC-3000 column oven (P/N 6011702)
- DAD-3000RS diode array UV/VIS (P/N 8122745)
- Analytical Flow Cell DAD-3000(P/N 4216/4043)

The UltiMate 3000 system was operated with Thermo Scientific™ Chromeleon™ SR4 software, version 7.2.

The preparative LC was an Interchim PF4250 unit operated with Interchim InterSoft® software version 5.1c.09.

Consumables

HPLC solvents and HPLC-grade water were from Fisher Chemical.

Columns

- Analytical, Thermo Scientific™ Hypersil GOLD™ C18, 5 µm, 4.6 × 250 mm (P/N 25005-254630)
- Preparative, Thermo Scientific™ Hypersil GOLD™ C18, 5 µm, 20 × 250 mm (P/N 25005-259270A)

Analytical conditions and method

Mobile phase A:	20 mmol ammonium acetate in water	
Mobile phase C:	Acetonitrile, HPLC gradient grade	
Gradient:	0 min	95% A, 5% C
	48 min	0% A, 100% C
	60 min	0% A, 100% C
	62 min	95% A, 5% C
	75 min	95% A, 5% C (equilibration for next injection)
Flow rate:	1.2 mL/min	
Run time:	75 min	
Column temp.:	20 °C	
Injection volume:	10 µL	
Injection conc.:	1 mg/mL	
Detection:	254 nm	

Preparative conditions and method

Mobile phase A:	20 mmol ammonium acetate in water	
Mobile phase B:	Acetonitrile, HPLC gradient grade	
Gradient:	0 min	95% A, 5% C
	48 min	0% A, 100% C
	60 min	0% A, 100% C
	62 min	95% A, 5% C
	75 min	95% A, 5% C
Flow rate:	18 mL/min	
Run time:	58.5 min	
Column temp.:	Ambient	
Injection volume:	1000 µL	
Injection total mass:	40 mg	
Detection:	254 nm	

Sample preparation and workflow

The overall workflow breaks down into several principal steps. First, the crude reaction material is injected into an analytical column setup. This allows investigation into the proper separation of the target components from educts, side products, or even contaminations. Typically, as applied in this case, a generic gradient profile is chosen to separate unknown or unexpected compounds from the target compound(s). Further, a long column is used, often 250 mm, to maximize peak capacity for this “pilot” run. In cases where the resolution is not good enough to trigger effective fractionation, the gradient profile or even buffer/solvent conditions must be adjusted (Figure 1).

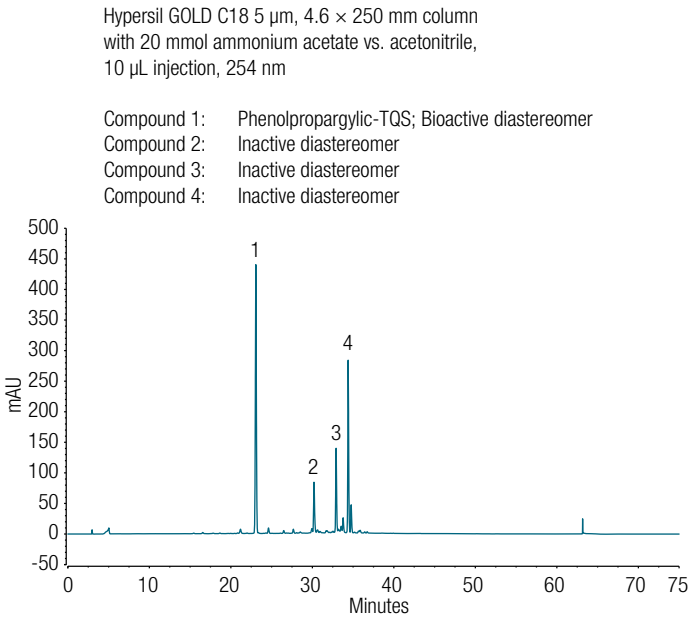


Figure 1. Pilot run on UltiMate 3000 RS Quaternary UHPLC system. This analytical chromatogram shows the crude sample before the preparative run, using conditions described in the experimental section, with return to the initial conditions.

Second, with the knowledge and potential adaptation of the separating method, the “pilot” run is upscaled to a larger diameter preparative column setup usually maintaining similar packing media, size, and chemistry to facilitate method transfer. Thermo Fisher Scientific provides an online calculator that assists with adjusting flow and gradient timings (if applicable) to the preparative column format chosen (thermofisher.com/PrepLCTool).

Though the preparative run often exceeds the absorption maxima of the UV detector used (mass on column and flow cell light path length dependent), the principal resolution between target compound(s) and unwanted educts and contamination needs to be maintained (Figure 2).

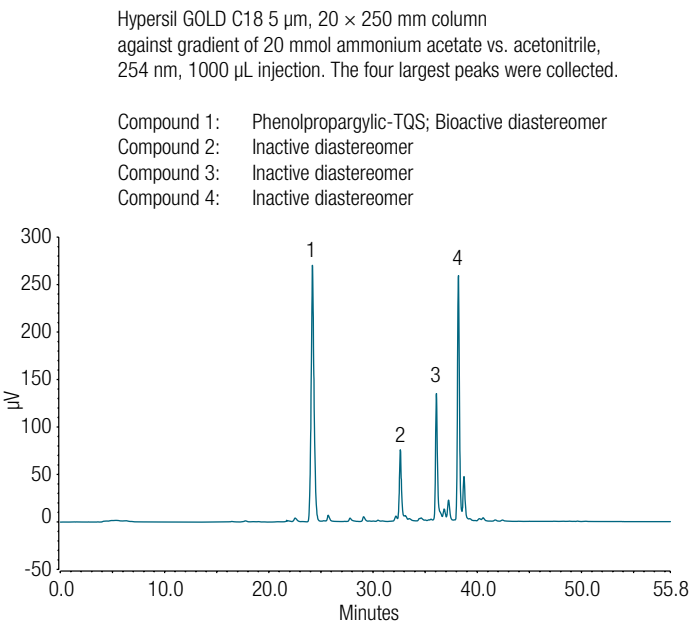


Figure 2. Preparative LC run on Interchim PF4250. Enlargement of the four diastereomers zone was observed.

The experimenter will aim to minimize sample consumption for the “pilot” experiments while maximizing yields in the following preparative run(s). In this case, the samples were prepared as follows:

Pilot run

- Target mass in sample 0.01 mg/mL (10 µL injected of a 1 mg/mL solution in acetonitrile / water = 5/5 (v/v)

Preparative run

- Target mass on column = 2 x 20 mg (injected in 1000 µL, 2 separate preparative runs, also acetonitrile / water = 5/5 v/v)

Often software can help to find reasonable fraction collecting parameters. A UV signal threshold can be set on the preparative LC system where all effluent out of the detector will be collected exceeding this threshold. The trigger can be programed to consider slopes of peak start or peak end, overrunning signal in the UV detector or noise management and baseline drift management.

Chromeleon CDS offers the capability to optimize fractionation settings (without any further sample loss) based on the chromatogram from one pilot run of the sample. If the upscaling remains within the linear range of the detector, these settings can be adjusted accordingly also for the preparative run.

Assess the quality of fractions

The last step for purifying target compounds is often related to the accomplished purity of the collected material. Typically, there are more offline tests included such as confirming the structure using NMR or mass confirmation using single quadrupole MS detectors (which could be also be an online detector in the analytical system setup).

Here we show the reinjection of the collected fractions in the analytical HPLC or UHPLC system to assess the quality (Figure 3). The chromatogram received is typically processed for peak area and the purity of the target compound calculated as percentage of the target peak over the overall integrated area of all found peaks.

Results

The Hypersil GOLD C18 columns chosen performed well for both the pilot run and subsequent preparative run. The overall retention between the two-vendor platform was maintained and the fractionation settings provided a very good purity for the target components. Scale-up was easy and straightforward.

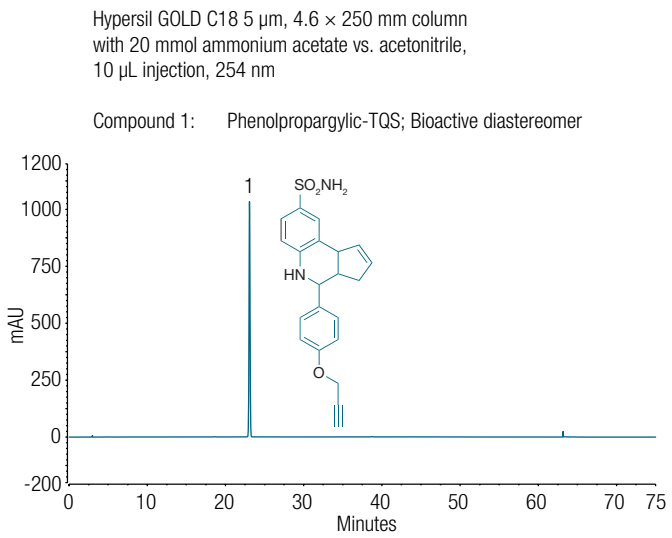


Figure 3. Purity check run of the main fraction for targeted phenolpropargylic-TQS on the UltiMate 3000 RS Quaternary UHPLC system (Fraction 1 isolated by preparative chromatography)

Discussion

The success and efficiency of a clean-up or purification procedure after synthesis is dependent on a successful pilot run.

Once the resolution is satisfactory and all isolation objectives are fulfilled, the pilot run can be easily scaled up to the preparative experiments without further loss of precious sample. While this is possible across different vendors' LC platforms, controlling fractionation and separation with the same chromatographic data system improves and enhances the overall workflow.

thermo scientific

Conclusion

In this example we demonstrate a solid, generically applicable workflow for bioorganic, medicinal, and synthetic chemists and other researchers. With Thermo Fisher Scientific now offering more column choices for preparative LC, scientists can easily scale up their chromatography using their preferred Thermo Scientific column brands.

The Hypersil GOLD C18 column used enabled the separation and resolution of four diastereoisomers, which are typically hard to resolve, in both the analytical and preparative formats. The Hypersil GOLD C18 proprietary ligand chemistry offers a robust, easy to scale up solution for more advanced purifications as demonstrated in this application note.

Links

COBRA – Laboratory

Preparative HPLC Method Transfer Tool

Thermo Scientific Preparative HPLC Columns

Chromeleon CDS and Fractionation

UltiMate 3000 RS Quaternary UHPLC

References

1. Thakur, G.A.; Kulkarni, A. R.; Deschamps, J.R.; Papke, R.L. Expeditious Synthesis, Enantiomeric Resolution, and Enantiomer, Functional Characterization of (4-(4-Bromophenyl)-3a,4,5,9btetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): An Allosteric Agonist-Positive Allosteric Modulator of α7 Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **2013,** *56,* 8943–8947.
2. Mercey, G.; Verdelet, T.; Renou, J.; Kliachyna, M.; Baati, R.; Nachon, F.; Jean, L.; Renard, P.-Y. Reactivators of Acetylcholinesterase Inhibited by Organophosphorus Nerve Agents. *Accounts of Chemical Research* **2012,** *45*(5), 756–766.

Find out more at **thermofisher.com/PrepLC**

ThermoFisher SCIENTIFIC