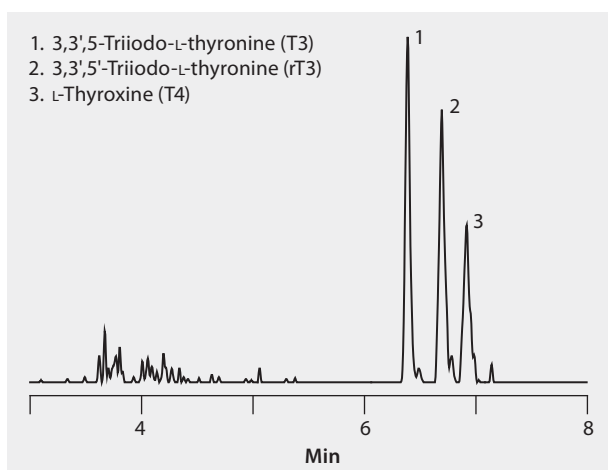


LC/MS with Online Solid Phase Extraction (SPE-LC/MS) of Thyroid Hormones in Plasma on Ascentis® Express 2.7 µm Phenyl-Hexyl using RP-Amide Traps

An online SPE-LC/MS method is shown here for the determination of thyroid hormones in blood plasma. The method exploits RP-Amide or C8 as the trapping column and a phenyl-hexyl phase as the analytical column, respectively. Under the optimized conditions, both RP-Amide and C8 effectively trapped the thyroid hormones extracted from spiked rabbit plasma sample which had been protein precipitated. However, the RP-Amide traps were shown to be superior to the commonly used C8 traps because they provided higher analyte signals and recoveries. Additionally, RP-Amide traps are more flexible with washing solvent as they are compatible with 100% aqueous mobile phases and can be used with up to 20% methanol with minimal sample loss. Separation was carried out using an Ascentis Express 2.7 µm Phenyl-Hexyl column. Fluka CHROMASOLV solvents provided clean, robust operation. Cerilliant CRMs provided reliable quantification.

market focus	Clinical; Life Science and Biopharma
sample preparation	SPE (Solid Phase Extraction)
column	Ascentis Express Phenyl-Hexyl, 5 cm x 2.1 mm I.D., 2.7 µm particles (53334-U)
mobile phase	[A] water; [B] methanol, both with 0.1% acetic acid
gradient	5% B for 3 min; to 70% B in 2 min, hold 3 min
pressure	1800 psi
column temp.	ambient
detector	MS, ESI(+), MRM mode (777.7/731.8 for T4; 651.8/605.5 for T3 or rT3)
injection	5 µL
sample	protein precipitated rabbit plasma spiked with desired amount of T4, T3 and rT3
Application No.	G006223



Related Products

- analytical column**
Ascentis® Express Phenyl-Hexyl, 2.7 Micron HPLC Column ([Supelco 53334-U](#))
- guard cartridge**
Ascentis® Express Guard Cartridge Holder ([Supelco 53500-U](#))
Ascentis® Express RP-Amide, 2.7 Micron Guard Cartridge ([Supelco 53514-U](#))
- mobile phase component**
Acetic acid ([Fluka 49199](#))
Methanol ([Fluka 14262](#))
Water ([Fluka 14263](#))
- sample prep**
EXP® Pre-Column Filter ([Supelco 51163-U](#))
EXP® Pre-Column Filter Cartridges ([Supelco 51166-U](#))
- standard**
L-Thyroxine (T₄) solution ([Cerilliant T-073](#))
3,3',5-Triiodo-L-thyronine (T₃) solution ([Cerilliant T-074](#))
3,3',5'-Triiodo-L-thyronine (Reverse T₃) solution ([Cerilliant T-075](#))