Featured Application: *Urinary Free Cortisol and Cortisone on Raptor Biphenyl*

LC-MS/MS Analysis of Urinary Free Cortisol and Cortisone without Matrix Interferences

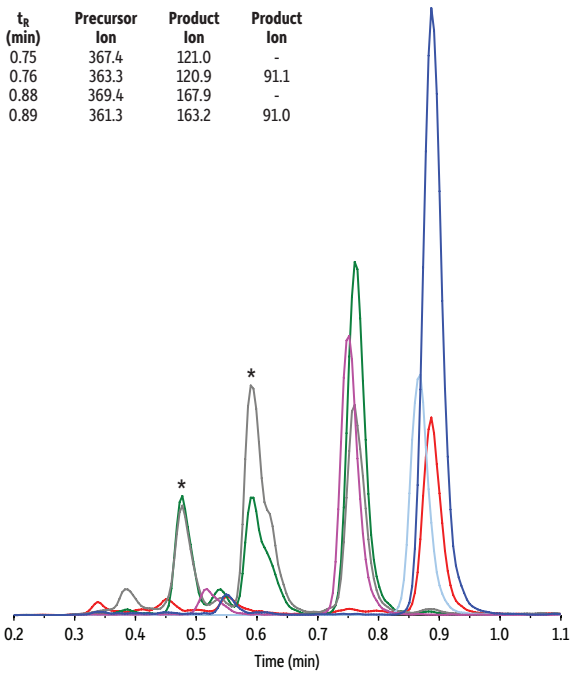
- Raptor Biphenyl column provides reliable separation of analytes from endogenous matrix interferences.
- Heightened selectivity for compounds that are hard to resolve on C18 and other phenyl chemistries enables accurate results.
- Fast 3-minute analysis allows high sample throughput.

Cortisol is a steroid hormone synthesized from cholesterol by a multienzyme cascade in the adrenal glands. It is the main glucocorticoid in humans and plays a critical role in glucose metabolism and immune response regulation. Cortisone, a downstream metabolite of cortisol, is measured in conjunction with cortisol to help diagnose various adrenocortical disorders, particularly hypercortisolism (Cushing syndrome). Urinary free cortisol and cortisone levels correlate well with bioactive plasma concentrations and, thus, are important biomarkers. LC-MS/MS is the preferred screening technique for these compounds because it is highly selective and helps eliminate analytical interferences that can affect immunoassay-based methods.

Due to the structural similarities between cortisol and cortisone and the potential for matrix effects from urine components, appropriate column selectivity is critical for obtaining accurate results. In the LC-MS/MS analysis of urinary free cortisol and cortisone developed here, both compounds were completely resolved from endogenous matrix interferences using a highly selective Raptor Biphenyl column. Chromatographic separation was essential in this case because some of the matrix compounds are isobaric to cortisol and cannot be distinguished by the mass spectrometer. Using a Raptor Biphenyl column and the conditions shown here, baseline separation was obtained in a 3-minute analysis, which allows labs to quickly generate more accurate quantitative data for clinical diagnosis.

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. Cortisol-d4	0.75	367.4	121.0	-
2. Endogenous cortisol	0.76	363.3	120.9	91.1
3. Cortisone-d8	0.88	369.4	167.9	-
4. Endogenous cortisone	0.89	361.3	163.2	91.0

*Isobaric matrix peaks



LC_CF0697

Column	Raptor Biphenyl (cat.# 9309A52)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	2.7 µm
Pore Size:	90 Å
Guard Column:	Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.:	40 °C
Sample	
Diluent:	Mobile phase A
Conc.:	Calculated concentration is 105.1 ng/mL and 135.9 ng/mL for cortisol and cortisone, respectively
Inj. Vol.:	10 µL
Mobile Phase	
A:	Water, 0.1% formic acid
B:	Acetonitrile, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	70	30
1.00	0.6	70	30
1.01	0.6	0	100
1.50	0.6	0	100
1.51	0.6	70	30
3.00	0.6	70	30

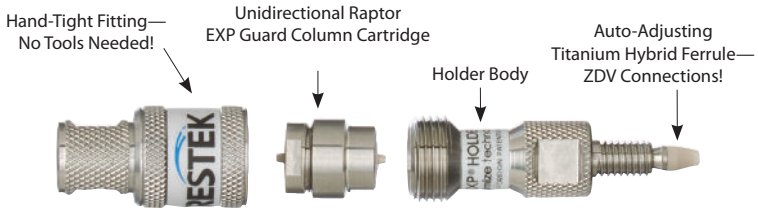
Max Pressure:	400 bar
Detector	MS/MS
Ion Mode:	ESI+
Mode:	MRM
Instrument	UHPLC

Notes: Sample Preparation Method: 1. Centrifuge female human urine for 5 min at 4,500 rpm, 10 °C 2. Aliquot 380 µL supernatant. Add 20 µL each of internal standard solution (1 µg/mL in methanol) 3. Load 200 µL of sample on to ISOLUTE SLE+ 200 µL supported liquid extraction plate (part# 820-0200-P01) 4. Apply a pulse of vacuum to initiate flow 5. Wait 5 min for sample to completely absorb 6. Extract samples with 1 mL of MTBE. Allow solvent to flow for 5 min under gravity. Apply vacuum for 10-30 sec to complete elution 7. Evaporate extracts to dryness under a stream of nitrogen 8. Reconstitute in 200 µL mobile phase A prior to analysis 9. Vortex to mix.

Raptor Biphenyl LC Columns (USP L11)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9309232	—	—
50 mm	9309252	930925E	—
100 mm	9309212	930921E	—
150 mm	9309262	—	—
2.7 µm Columns			
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65
5 µm Columns			
30 mm	—	930953E	—
50 mm	9309552	930955E	9309555
100 mm	9309512	930951E	9309515
150 mm	9309562	930956E	9309565
250 mm	—	—	9309575

Raptor EXP Guard Cartridges



Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

EXP Direct Connect Holder

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Maximum holder pressure: 20,000 psi (1,400 bar)

Raptor EXP Guard Column Cartridges

Description	Particle Size	qty.	5 x 2.1 mm cat.#
Raptor Biphenyl EXP Guard Column Cartridge	2.7 µm	3-pk.	9309A0252

Maximum cartridge pressure: 600 bar/8,700 psi (2.7 µm) or 400 bar/5,800 psi (5 µm).
Raptor SPP LC columns combine the speed of SPP with the resolution of USLC technology.
Learn more at **www.restek.com/raptor**

Hybrid Ferrule U.S. Patent No. 8201854, EXP Holders U.S. Patent No. 8696902, EXP2 Wrench U.S. Patent No. D766055. Other U.S. and Foreign Patents Pending. The EXP, Free-Turn, and the Opti- prefix are registered trademarks of Optimize Technologies, Inc.

Resprep VM-96 Vacuum Manifold

for 96-Well Plates

Description	qty.	cat.#
Resprep VM-96 vacuum manifold	ea.	25858

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