

Enabling Low Level LC/MS Measurement of Analytes in Urine with Online SPE

Many common LC/MS assays in the Clinical laboratory involve the analysis of various intrinsic compounds or xenobiotic drugs from urine. While typically free of components such as physiological proteins that can interfere with LC/MS analysis, urine contains a relatively high concentration of salts and other ions that can interfere with LC/MS. These salts can cause potential problems in an LC/MS assay by interfering with chromatography or causing ion suppression or background interference in MS detection.

One common technique to avoid some of the analytical problems caused by urine samples is to prepare samples for LC/MS by Solid Phase Extraction (SPE). Use of SPE allows analytes of interest to be separated from interfering salts and other ions and help enable low levels of detection with MS. In this study, the effects of using the Masstrak™ Online SPE Analyzer for the LC/MS analysis of an analyte in urine is presented.

- Improve laboratory productivity with integrated sample preparation and LC/MS analysis
- Increase LC/MS assay sensitivity
- Decrease background interference and ion suppression effects



MassTrak Online SPE Analyzer. This system includes an ACQUITY UPLC®, ACQUITY® TQ Detector, and an ACQUITY UPLC Online Sample Manager.

Removal of Urine Salts by Online SPE

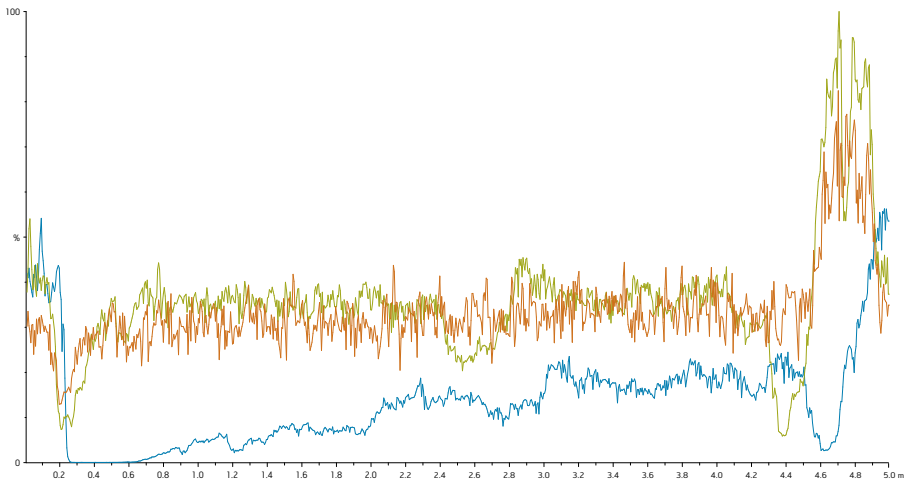


Figure 1: Removal of salts by Online SPE. MS signal from water (red) compared with MS background signal generated from urine (blue) and urine treated by online SPE (green). Note the significant decrease in background signal of the urine sample after SPE treatment..

Analysis of Low Levels of Cortisol in Urine using Online SPE

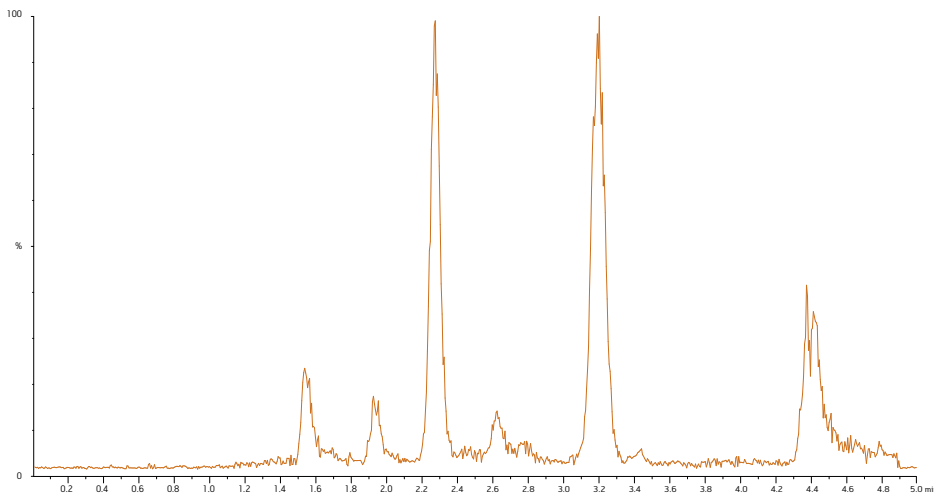


Figure 2: LC/MS analysis of 5 ng/ml urinary free cortisol in urine diluted 1:4 and analyzed using the Masstrak Online SPE Analyzer. The cortisol peak is present at the 3.2 minute retention time. Other peaks are isobaric interferences.

Calibration Curve of Urinary Free Cortisol

Compound name: Cortisol (1)
Correlation coefficient $r = 0.999749$, $r^2 = 0.999499$
Calibration curve: $0.319462 * x + -0.0552005$
Response type: Internal Std (Ref 2), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

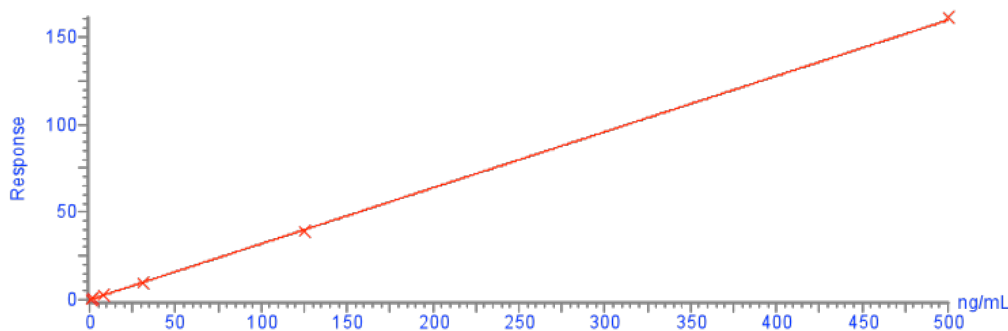


Figure 3: Analysis of urinary free cortisol using the MassTrak Online SPE Analyzer. Note the dynamic range for this analysis ranges from low ng/ml to hundreds of ng/ml and covers more than two orders of magnitude dynamic range.

CONCLUSION

This study has determined that by using the MassTrak Online SPE Analyzer, analysts are able to successfully measure low levels of an analyte (urinary free cortisol) from a urine sample. The MassTrak Online SPE Analyzer is capable of dramatically reducing the background signal from the urine sample matrix. Removal of these interfering compounds allowed accurate measurement of an analyte of interest down to a level of 5 ng/ml. This kind of LC/MS assay sensitivity would be difficult without SPE sample preparation.

Furthermore, in this case, the analyte of interest was analyzed in about 3-5 minutes/sample. This fast elution time, when coupled with the online SPE capabilities of this system, could be utilized to analyze a large number of urine samples quickly and efficiently.

ORDERING INFORMATION:

MassTrak Online SPE Analyzer (Part Number 176015065IVD) includes:

ACQUITY UPLC Sample Manager (Part Number 186015006IVD)

ACQUITY UPLC Binary Solvent Manager (Part Number 186015001IVD)

ACQUITY UPLC Column Manager – A (Part Number 186015043IVD)

ACQUITY® TQ (Part Number 186003875IVD) or XEVO® MS Detector

ACQUITY UPLC Online SPE Manager (Part Number 186015012IVD)

Description	Part Number
MassTrak C ₁₈ OSM, 10 µm, 1x10 mm, 96/pk	186005672IVD

REFERENCE:

¹Data available in Eastwood et al. Ann Clin Biochem 49:110 Suppl 1. and is also on file at Waters.

FOR IN VITRO DIAGNOSTIC USE.

The MassTrak Online SPE Analyzer may not be available in all countries. For more information contact your local Waters representative.

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