An Automated Procedure for Determining ITSP SPE Analytical Parameters for the Analysis of Cortisol and Cortisone in Urine

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Overview

•A CTC Analytics HTS sample handler was used to extract and clean-up urine samples using ITSP SPE devices and inject the extracts onto LC/MS/MS.

•This method uses only standard laboratory equipment.

•A fully automated method has been developed for the optimization of the ITSP SPE extraction.

•This procedure eliminates most of the manual sample manipulation found in the traditional assay development.

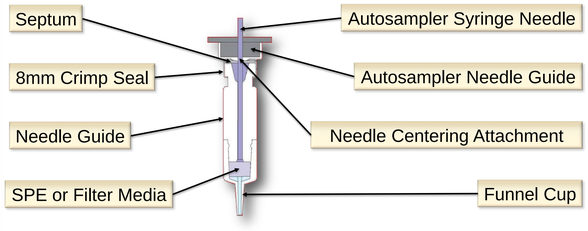
Introduction

ITSP (Instrument Top Sample Prep) is a consumable device that allows for a common autosampler used on most chromatographs to prepare client samples using Solid Phase Extraction (SPE) or filtration.

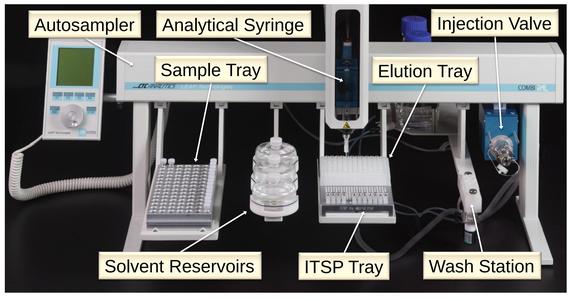
ITSP's septum seals and grips the needle creating a closed environment above the bedmass. By removing the volume above the column bedmass and replacing it with the analytical instrument's syringe, solutions can be passed across the bedmass using the hydraulic pressure of the syringe plunger. The gripping action of the septum also allows for the ITSP device to be transported to any location on the autosampler. By defining waste and elution locations the device can be prepped in one location and moved to elute the collected compounds in another. Once eluted into a clean receptacle the instrument can discard the device and inject the eluate onto the analytical instrument.

The following diagrams illustrate the ITSP device and the typical set-up on an LC/MS/MS autosampler.

ITSP Device Components

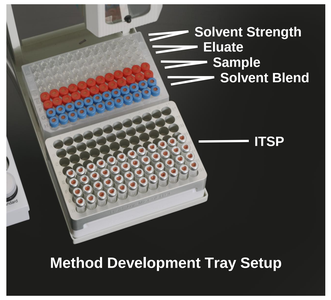


ITSP Autosampler Setup

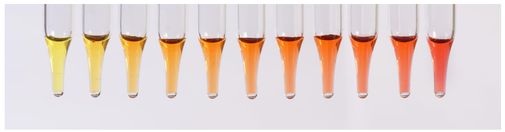


Automated Method Development

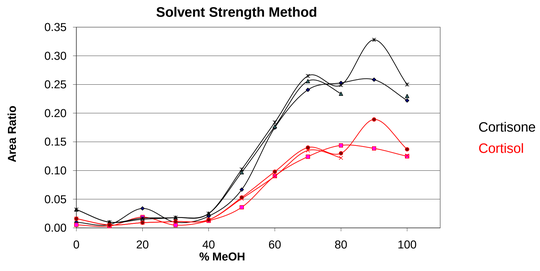
MicroLiter has developed a 96 well microplate and a set of macros that automates method development based on application notes provided by most sorbent manufacturers. It is also an excellent tool for scaling down methods you believe can increase the productivity of your lab while at the same time reducing the costs of solvents, raw sample requirements and hazardous waste disposal.



Method Development Tray Setup

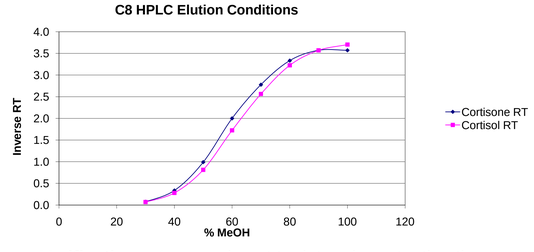


Step 1. Blend Solvents – The macro ***ITSP SPE MD Blend Solvents*** blends two solvents beginning with 100% in position one decreasing to 0% in position 11. Likewise Solvent two starts with 0% in position 1 and ends with 100% in position 11.



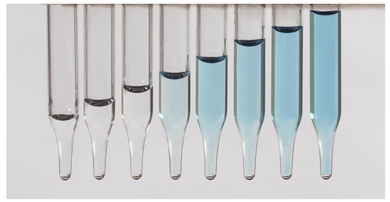
Step 2. Solvent Strength – The macro ***ITSP SPE MD Solvent Strength*** simply loads the device with sample and elutes with the blended solvents. As illustrated, the sample begins to elute at approximately 37% Methanol, which yields the optimum wash solvent strength.

At approximately 70% Methanol the sample elution is complete, which yields the optimum elution solvent strength, and both parameters are set. One other note: One of the chromatograms was produced using an ITSP device twice proving that ,in situations where chain-of-custody is not a factor, the device can be reused.

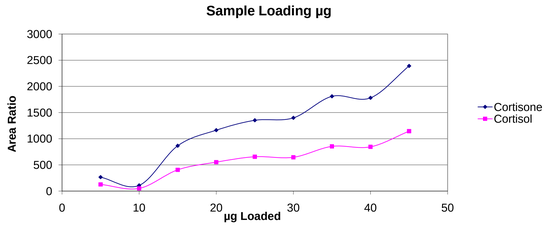


Verification – An experiment to determine retention times for the test compounds on a C8 HPLC column was performed to verify the data from the solvent strength experiment.

Step 3. Sample Breakthrough - The macro ***ITSP SPE MD Sample Loading Capacity*** simply loads the ITSP device with sample of increasing volumes in increments of 10µL until breakthrough occurs. In this pictorial representation, the blue food coloring begins to breakthrough at between 30 and 40µL. If the sample is relatively clean, then direct injection without a wash can offer specific data for sample breakthrough.



However, if the sample is dirty it could clog the orifice or damage the analytical column, so the device would require regular SPE wash and elution steps, as in Step 4 below.



Step 4 - This chart illustrates the 10mg C8 sorbent had no issues loading the sample within the requirements of the assay using the macro ***ITSP SPE MD Full Load Wash Elute Capacity***.

Methods

Sample Preparation Method

A standard of Cortisol and Cortisone was prepared in synthetic urine[1] at a nominal concentration 50 ng/mL for the solvent strength and C8 HPLC elution experiments and at 50 µg/mL for the sample loading experiment. Cortisol stable label internal standard (50 ng) was added to the eluant wells. The sample preparation and method development macros written by MicroLiter were independently tested and verified by Thurman Allsup at OpAns LLC.

Extraction Hardware

ITSP Cartridges: C8 10 mg (MicroLiter Product No.: 07-C810-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µl syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW).

ITSP SPE Find Solvent Strength Method

Step	Solvent	Volume (µL)	Flow Rate (µL/sec)
Condition	B	100	15
Condition	A	100	15
Load	Sample	100	10
Flush	Air	100	15
Elute	C	100	5
Flush	Air	100	40

Solvent A: Water
Solvent B: Methanol
Solvent C: Methanol:Water (A gradient of mixtures from100% methanol to 100% water in 10% increments)

C8 HPLC Elution Conditions

A Zorbax Eclipse XDB-C8 HPLC column with a flow of 0.6 mL/min was used to determine retention times for cortisol and cortisone while varying isocratic mobile phases in 10% increments from 30-100% methanol and water.

ITSP SPE Find Sample Loading Method

Step	Solvent	Volume (µL)	Flow Rate (µL/sec)
Condition	B	100	15
Condition	A	100	15
Load	Sample	100-900	10
Flush	Air	100	15
Elute	C	100	5
Flush	Air	100	40

Solvent A: Water
Solvent B: Methanol
Solvent C: Methanol:Water (A gradient of mixtures from100% Methanol to 100% water in 10% increments)

Analysis Method

All analysis was on a HPLC/MS/MS using a Finnigan TSQ Quantum Ultra AM triple quadrupole with a Positive Ion (HESI) Heated Electrospray Ionization Probe and an Agilent 1200 Rapid Resolution HPLC. Area ratios of compound area to internal standard area were calculated.

Results

Using ITSP SPE method development macros for automating sample preparation, in less than an hour it has been shown that the optimum wash solvent is 30-40% methanol in water and the optimum elution solvent is 70-80% methanol in water. The experiment determining retention times for the test compounds while changing mobile phases confirmed that the test compounds just started to elute at 30-40% methanol and were eluted quickly at 80-90% methanol. Using the sample load macro, in less than an hour it was determined that the sample capacity was above 45 µg in 900 µL urine.

Conclusion

One of the advantages of ITSP SPE is the ability to perform automated sample extraction, cleanup, and extract injection in-line. A simplified method for the optimization of an ITSP SPE method has been demonstrated here.

References

1. McCurdy D, Lin Z, Inn KGW, Bell R, Wagner S, Efurd, DW, Steiner R, et al. Second interlaboratory comparison study for the analysis of ^{239}PU in synthetic urine at the µBq (~100 aCi) level by mass spectrometry. *Journal of Radioanalytical and Nuclear Chemistry.* 2005;263/2:447-445.

For Further Information

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