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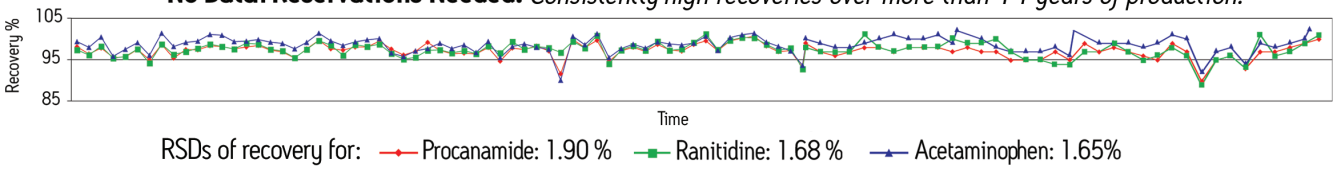
OASIS SAMPLE EXTRACTION PRODUCTS: FREQUENTLY ASKED QUESTIONS

Q. Do Oasis® solid-phase extraction (SPE) products suffer from batch-to-batch reproducibility issues as other sorbents have?

A. Waters specification for batch-to-batch variability is <5%. However, over the past 14 years, Oasis products consistently have a reproducibility of <2% RSD, and this is measured by the reproducibility of the recovery of three model analytes.

No Batch Reservations Needed: *Consistently high recoveries over more than 14 years of production.*



Q. Do I need to reserve an Oasis SPE batch to ensure my assay reproducibility?

A. No, as shown in the graph above, Oasis SPE offers great batch-to-batch reproducibility that should provide you with full confidence that all Oasis SPE batches will perform similarly throughout the life of the assay.

Q. Does Oasis sorbent generate any leachable material that may impact the MS analysis?

A. No, Oasis sorbent does not leach any materials that impact the MS analysis or reduce the MS response.

Q. How do I process Oasis products in its various formats?

A. All Oasis products may be processed by positive pressure, centrifugation, or vacuum pressure. The centrifugation requires a deep enough open swing bucket that can hold the 96-well plate and the collection plate. Waters offers a vacuum manifold and a pump, and recently introduced the Waters Positive Pressure-96 Processor that provides highly uniform flow from well-to-well, and superior flow for viscous samples.

Q. Do your Oasis 96-well or µElution plate formats work with my automated liquid handler?

A. All Oasis 96-well and µElution plates are configured properly to fit and perform with all available SPE automation and liquid handling processers.

Q. What about drying out of the Oasis sorbent bed during processing?

A. Oasis is a water-wettable polymeric sorbent that been designed to perform even if the sorbent bed becomes dry. It retains its solvated state throughout the extraction process. Oasis sorbent does not suffer from the dewetting challenges present in bonded silica sorbents.

Q. Do I need to condition my Oasis sorbent?

A. No. However, while Oasis is a water-wettable sorbent and does not require conditioning before sample loading, we recommend a conditioning step to eliminate any impact of the elution solvent/sorbent interaction on the assay results.

Q. Can I isolate an analyte and a metabolite at the same time using the Oasis sorbent?

A. Yes. However, care needs to be taken when selecting the sorbent and the protocol to make sure that none of the modifiers used convert the metabolites back to the analyte. Also, all the solutions used for the wash and elution steps should impact both the analyte and metabolite(s) in the same manner.

Q. How much plasma can I load onto 1 cc and 3 cc cartridges, 96-well plates, and µElution plates, and what are the wash and elution recommended volumes?

A. The following table will provide the volume recommendations for the various format sizes used in bioanalysis.

Recommended Volume for Generic Methods (assuming 1:1 dilution)

	Cartridges		96-Well Plate				µElution Plate
Cartridge size/sorbent mass	1 cc	3 cc	5 mg	10 mg	30 mg	60 mg	2 mg
Condition/ equilibration (mL)	1mL	2 mL	0.2 mL	0.5 mL	0.5-1mL	0.5-2 mL	0.2 mL
Load (mL urine or plasma*) (total of matrix and dilution)	1mL	2 mL	1 mL	1-2 mL	1-2 mL	1-2 mL	Up to 0.75 mL
Wash (mL)	1mL	2 mL	0.2 mL	0.5 mL	0.5-1 mL	1-2 mL	0.2 mL
Elute (mL)	1mL	2 mL	0.05-0.2 mL	0.15-0.3 mL	0.4-1.0 mL	0.8-2 mL	0.025-0.10 mL

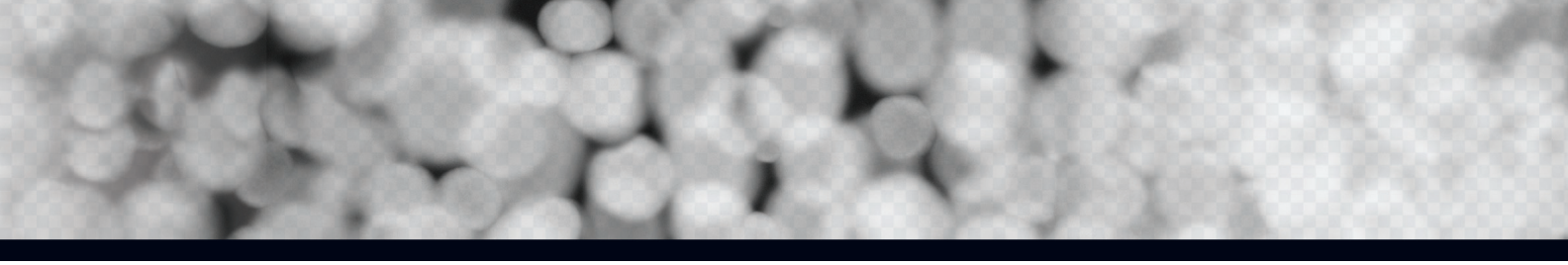
Note: Above listed sample volumes are for biological samples.
•Solution should be made fresh daily.

Q. Are there pH or solvent limitations for the Oasis sorbents?

A. As a polymeric resin, Oasis sorbents can perform over an extended pH range of 0-14, where bonded silica sorbents are traditionally limited to a pH range of 2-8. Also, there is no solvent limitation observed or reported with any solvent typically used in bioanalysis.

Q. If I want to transfer a reversed-phase method from a C_{18} bonded silica to Oasis HLB, what should I consider?

A. Oasis HLB sorbent has a relative hydrophobic retention capacity 3X higher than that of traditional silica-based SPE sorbents like C_{18}. This means that similar performance can be obtained with 1/3 of the sorbent bed mass of a C_{18}, and use commensurately smaller wash and elution volumes.



Q. What should I consider when selecting a collection plate for the µElution format?

A. Since the objective in using the µElution format is to generate as small an elution volume as possible, the main consideration for choosing a collection plate should be the residual volume. It is important to use a collection plate that has minimal residual volume because µElution plate elution volumes can be a low as 25 µL. The ACQUITY 1 mL collection plate (P/N 186002481) is ideal as it has a residual volume of only 15 µL.

Q. What is the capacity of the µElution plate (i.e. How much of my compound can I load onto the device)?

A. The capacity of the µElution plate is related more to the mass of interferences than the mass of compound as interferences tend to be present in very high concentrations relative to the concentration of the analytes. Interferences may interact by ion-exchange or reversed-phase retention and could use up available sites on the sorbent, leaving less capacity for the analytes. For example, in plasma, phospholipids are often present at mg/mL levels, while the analytes may be present in only ng/mL quantities. The interferences tend to be present in much higher concentrations than the compounds of interest. It is similar for urine, where salts, urea, and other ionic species may be present in very high concentration relative to the analytes and may "use up" available sites for ion-exchange interaction, leaving less sites/capacity for the analytes to bind by ion exchange. Typically, we think in terms of volumes of matrix, knowing that the capacity in each retention mode (reversed phase or ion exchange) will be different for each matrix. In many cases, one should be able to load about 300 µL-350 µL (600-700 µL diluted) undiluted plasma easily. For urine, if ion-exchange mechanism is utilized for retention, the capacity for the analytes may be slightly lower due to the highly ionic nature of the urine interferences. These are guidelines only, we should keep in mind that one should determine the capacity experimentally in all cases.

Q. How important is flow rate when using Oasis products?

A. Flow rate is a critical consideration for all SPE devices, including Oasis. This is particularly critical during the load and elution steps. The use of the slowest flow rate possible during the load step ensures enough contact time between the analyte and sorbent. This will allow partitioning between the phases and for analyte retention onto the sorbent to occur. In the elution step, the slow flow rate ensures that the elution solvent has sufficient residence time to penetrate into the sorbent pores and effectively elute all the analytes from the sorbent. A one drop per second or less is a favorable flow rate for the loading and elution steps for Oasis devices.

Q. What are the key properties of the Oasis base particle that make it desirable for use in the solid-phase extraction of therapeutic peptides?

A. The fact that Oasis is polymer-based limits any secondary silanol interactions; it is water wettable and stable across the extremes of pH.

Q. How do I keep track of the ionization states of the peptide and sorbent throughout the protocol, from sample pretreatment to the elution step?

A. It is easy to determine the ionization state of the sorbent, it has 1 pK_a and we know the pH ranges over which it is charged or not. It is not as straightforward with peptides. They have pIs, not pK_as, so there are always multiple charges on the compound, and multiple pK_as. In addition, all of the various amino acid side chains can interact in different ways with the sorbent under different conditions. This is why it is strongly recommended to use the therapeutic peptide screening protocol to determine the best sorbent and protocol to be used for a particular peptide, since it can be very difficult to predict the peptide behavior.

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