Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

OASIS ON-LINE COLUMNS SPE COLUMNS FOR LC/MS/MS

I. INTRODUCTION

The Oasis® on-line columns make it possible to analyze a specific analyte in a sample matrix with combined with appropriate Waters narrow-bore analytical columns (such as XBridge™, SunFire™, Atlantis®, XTerra® or Symmetry® columns).

CONTENTS

OASIS®
SAMPLE EXTRACTION PRODUCTS

a. Oasis Patented Chemistries for Oasis On-line Columns

There are five available Oasis sorbent chemistries which are designed to meet just about all of your on-line LC/MS/MS needs. They are all built upon unique water-wettable Oasis HLB copolymer and provide exceptional results. The sulfonated and Quanternary amine derivatives Oasis MCX and MAX are designed for selective retention of basic and acidic compounds respectively. Oasis WAX and WCX are the derivatives of Oasis HLB. These sorbents are specifically designed to offer the same benefits and features as the HLB with the ability to retain and release strong acids (e.g. sulfonates) and strong bases (e.g. quaternary amines), respectively.

Oasis HLB
Hydrophilic-**L**ipophilic **B**alance reversed-phase sorbent

Oasis MCX
Mixed-mode: **C**ation-e**X**change and reversed-phase sorbent

Oasis MAX
Mixed-mode: **A**nion-e**X**change and reversed-phase sorbent

Oasis WCX
Mixed-mode: **W**eak **C**ation-e**X**change and reversed-phase sorbent

Oasis WAX
Mixed-mode: **W**eak **A**nion-e**X**change and reversed-phase sorbent

b. Oasis On-line Column Configuration

There are three Oasis on-line column configurations to fit your specific needs:

1. The Oasis cartridge column fits into a Sentry™ holder that features a hand-tighten fitting for fast and convenient cartridge replacement.
2. The direct connect column can be screwed directly into the switchingvalve or connect to fittings like a conventional HPLC column.
3. The Oasis column features traditional HPLC column fittings and hardware.

All of these formats are available with the five Oasis sorbents and a choice of particle sizes. The enclosed Certificate of Analysis (C.O.A.) displays results from stringent quality control tests on the batch of polymer sorbent.

The analysis is typically carried out using two pumps. The first is an isocratic pump used as a standalone (no need for additional software control) and the second is a binary pump used for elution. Generally, the extraction of a drug is carried out on a stationary phase with large particle (e.g. >25 µm) at high aqueous flow rate (4 mL/min). This technique separates the large molecules (i.e. proteins and other endogenous interferences), while the principal analyte is trapped on the reversed phase. A mass spectrometer in the multiple reaction monitoring (MRM) mode maximizes selectivity and sensitivity. It is recommend to add a simple protein precipitation step prior to injection. The Oasis on-line columns can give lifetime well over 100 injections.

II. SAMPLE PREPARATION

The plasma sample is prepared by mixing equal volumes of plasma with acetonitrile. Additives such as formic acid or ammonium hydroxide may be added at 2 % V/V to release the protein binding effect of plasma. An internal standard may also be added during this step at the appropriate concentration. The samples are vortexed and centrifuged at 3000 RPM for less than 30 minutes. This will force the precipitate transferred into a clean 96-well collection plate or vial, to the bottom of the collection vessel. Alternatively, with the assistance of a robotic liquid handler, the supernatant can be transferred to 96-well plates or vials.

III. EQUIPMENT

An HPLC system with minimum gradient mixing volume, and a sample management system with low carry over characteristics such as the Waters Alliance® HT system, is essential for rapid throughput. A switching valve is needed to divert interferences to a waste line. While the stand alone pump loads the cartridge at 4 mL/min, the binary pump keeps a constant and optimized 0.4 mL/min flow through the analytical column and the electrospray interface. There is no need to use a flow splitter.

NOTE: The maximum operational backpressure for these columns is 6,000 psi. Should you need to work at a higher pressure, please refer to the Oasis Direct Connect HP 2.1 x 30 mm Column for Automated Sample Preparation with Waters UPLC.

IV. TYPICAL CHROMATOGRAPHIC CONDITIONS

A very fast gradient is run with a total run time, including re-equilibration, of 3.0 minutes. The gradient profile is shown below (Table 1) where mobile phase B is 100 % aqueous with 0.5 % formic acid and mobile phase A is 100 % acetonitrile with 0.5 % formic acid. These mobile phases are recommended for the analysis of basic drugs. For acidic drugs, ammonium hydroxide is used instead. The switching valve diverts the cartridge effluent to waste for the first minute to prevent proteins and other endogenous interferences from entering the ESI interface. The valve is switched after one minute to redirect the flow to the MS. The gradient is carried out at a 0.4 mL/min flow rate. The injection volume of diluted plasma can be as low as 10 µL up to 200 µL depending on the required sensitivity. The expected lifetime is 200 injections with a 200 µL injection volume.

Table 1: Oasis HLB Gradient Method

Time (min)	HPLC Gradient Flow 0.4 mL/min		Valve Position
	A	B	Function
0.0	5	95	Load Position
0.5	5	95	
1.0	95	5	
2.6	95	5	Elution Position
2.9	95	5	
3.0	5	95	Equilbration/Load

A: Acetonitrile + 0.5% formic acid or 0.5% NH_4OH

B: Water + 0.5% formic acid or 0.5% NH_4OH

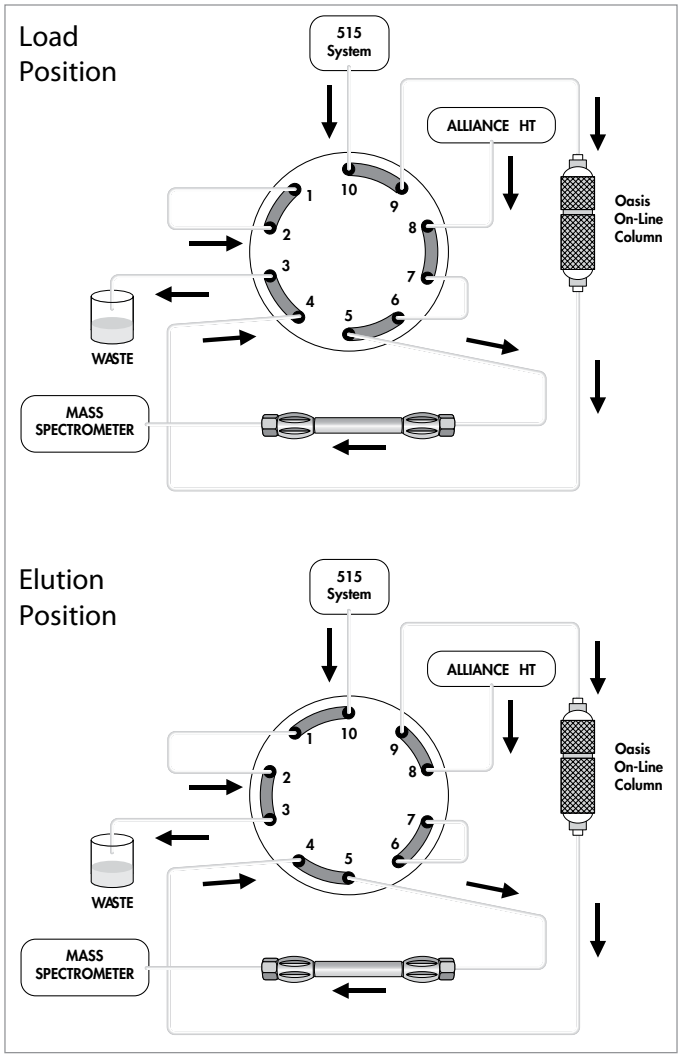

Figure 1: 10 Port Valve Setup for On-line SPE-LC/MS/MS

Sales Offices:

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 431 877 18 07

Australia 2 9933 1777

Belgium 32 2 726 1000

Brazil 55 11 5094 3788

Canada 800 252 4752

China 8621 6495 6999

CIS/Russia +7 495 3367000

Czech Republic 42 02 617 11384

Denmark 45 46 59 8080

Finland +358 9 5659 6288

France (33) 1 30 48 72 00

Germany 49 6196 400600

Hong Kong 852 29 64 1800

Hungary 36 1 350 5086

India and India Subcontinent 91 80 2 837 1900

Ireland 353 1 448 1500

Italy 02 265 0983

Japan (81) 3 3471 7191

Korea (82) 2 820 2700

Mexico 5255 5200 1860

The Netherlands +31 (0)76-50 87 200

Norway 47 63 84 60 50

Poland (48) 22 833 4400

Puerto Rico 787 747 8445

Singapore 65 6273 1221

Spain 34 93 600 93 00

Sweden 46 8 555 11500

Switzerland 41 56 676 7000

Taiwan 886 2 2543 1898

United Kingdom 44 208 238 6100

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

December 2012 715001394 RevD KK-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com