On-Line Extraction of Drugs and Their Metabolites from Biological Matrices using Strata™-X Extraction Cartridges Prior to LC/MS Analysis

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Abstract

While off-line solid phase extraction (SPE) continues to be a popular sample purification technique for drugs in biological matrices, it is not user friendly for high throughput screening. The recently introduced on-line extraction methods enable automation and integration with LC/MS, while permitting simultaneous extraction and concentration from matrix components. In this study, we introduce a new extraction cartridge for on-line SPE, which uses a functionalized polymeric phase, Strata™ X. This sorbent can effectively retain strongly polar and hydrophobic drugs under on-line extraction conditions. Such retention facilitates a stronger organic wash to remove matrix components which cause undesirable effects such as ion suppression during ESI-MS analysis. Employing seven drugs of widely different polarities, we demonstrate excellent recoveries and linearity over a wide range of concentrations. Consistent and reproducible performance is obtained over several hundred injections with direct plasma injections. The extraction of a drug/metabolite combination from plasma is also demonstrated.

Introduction

The tandem of liquid chromatography/mass spectrometry has become the principal mode of pharmaceutical analysis, especially for high throughput analysis employed in DMPK studies. Rapid analytical data generation is pivotal during these early stages of drug discovery. Analytical chemists have to deal with matrices such as plasma, serum, urine or even whole blood, requiring time consuming sample preparation/clean-up prior to LC/MS analysis meant to eliminate matrix components which cause rapid decline in HPLC column performance and have deleterious effects on quantitative analysis. Solid phase extraction (SPE) is the preferred sample pretreatment technique since it is amenable to automation, more efficient and less time/labor intensive of all sample preparation methods. However, the constant push for faster analysis times has stimulated investigation in alternative, more rapid approaches to sample preparation.

Introduction (cont.)

A viable, and increasingly popular alternative to off-line SPE is to perform the sample clean-up on-line, coupled to LC/MS which facilitates complete automation of the whole operation of pretreatment and analysis. In this study, we introduce a new extraction cartridge for on-line SPE, which uses a patent-pending functionalized polymeric phase, Strata™ X On-Line. This sorbent can effectively retain strongly polar and hydrophobic drugs under on-line extraction conditions. Employing seven drugs of widely different polarities, we demonstrate excellent recoveries and linearity over a wide range of concentrations, with consistent and reproducible performance over several hundred direct plasma injections. The extraction of a drug/metabolite combination from plasma is also demonstrated.

Materials and Methods

Instrumentation and Software:

HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA)
Quaternary pump(1), Binary pump(2), In-line degasser, DAD detector,
Autosampler, HP Chemstation software (Version A.09.01)
Phenomenex Synergi 10-port, 2-position switching valve
Bruker Esquire 2000 Ion-Trap MS analyzer

Columns:

Strata-X On-line 25µm SPE extraction cartridge (20x2.0mm) (00M-S033-B0-CB)

Luna C18(2) (50x4.6mm) (00B-4252-E0)

Luna NH_2 (50x4.6mm) (00B-4378-E0)

Synergi Hydro RP (50x4.6mm) (00B-4375-E0)

Strata™-X On-Line Extraction Cartridge

Strata™ –X, 25µ, 20x2.0mm, On-Line Extraction Cartridge Column

Novel, Patent-pending SPE Sorbent Technology in a Specialized On-Line Format

Offering:

- **Near Universal Selectivity** yielding high recovery (≥90%) for both highly polar and hydrophobic compounds
- **>200 direct plasma injections** per cartridge
- **Fast cycle times** for high through-put analysis
- Excellent reproducibility and high capacity

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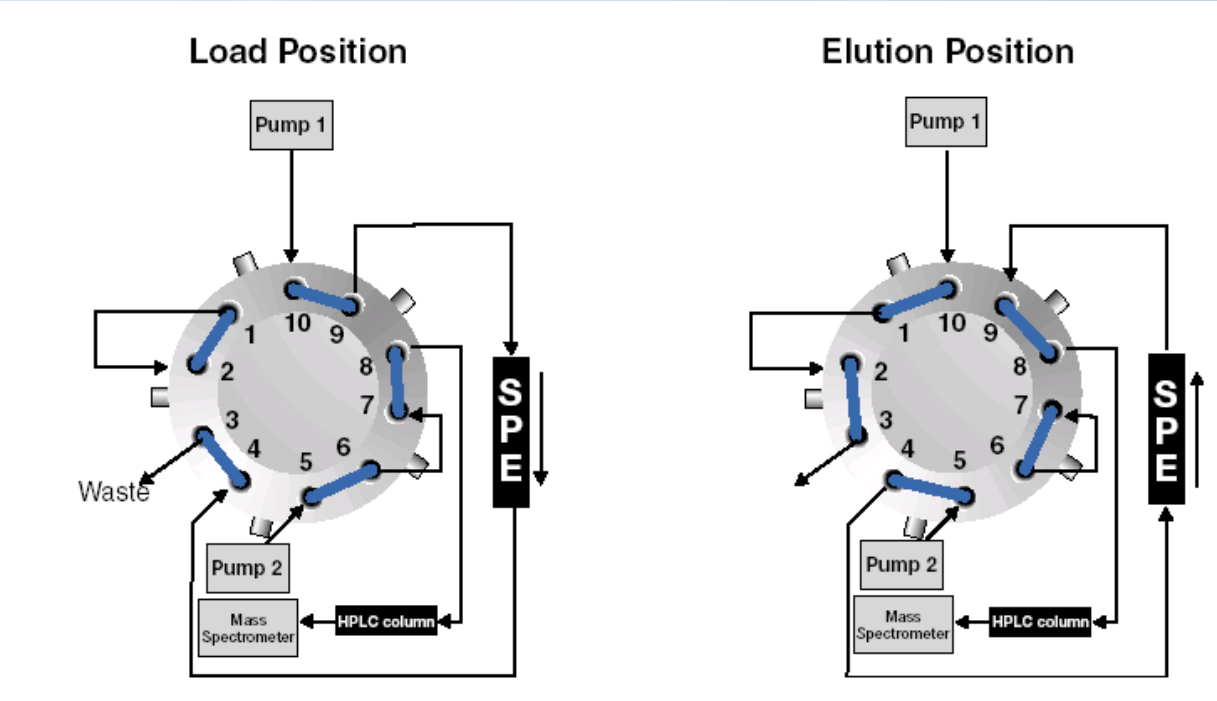
Valve Configuration for On-line SPE-LC/MS



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On-line SPE and HPLC Experimental Conditions

Table 1.

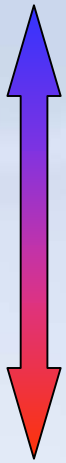
Time	HPLC Conditions		Valve Position
	Pump 1 (3mL/min)	Pump 2 (1mL/min)	
0	95%(A):5%(B)	95%(A):5%(B)	Load position
0.5		95%(A):5%(B)	Switch position Load to Elute
2.8		15%(A):85%(B)	Switch position Elute to Load
3	95%(A):5%(B)	95%(A):5%(B)	Load position

Mobile Phase:	A: 0.1% Formic Acid:H2O B: 0.1% Formic Acid:ACN
Injection:	50µl of diluted porcine plasma (1:1)
Detection:	Bruker Esquire 2000 Ion-Trap MS analyzer
Source:	ESI-Positive mode
Scan Range:	m/z 100-500
Run Time:	3 minutes (with analytical column)
Run Time:	1.2 minutes (without analytical column) (1 minute gradient)

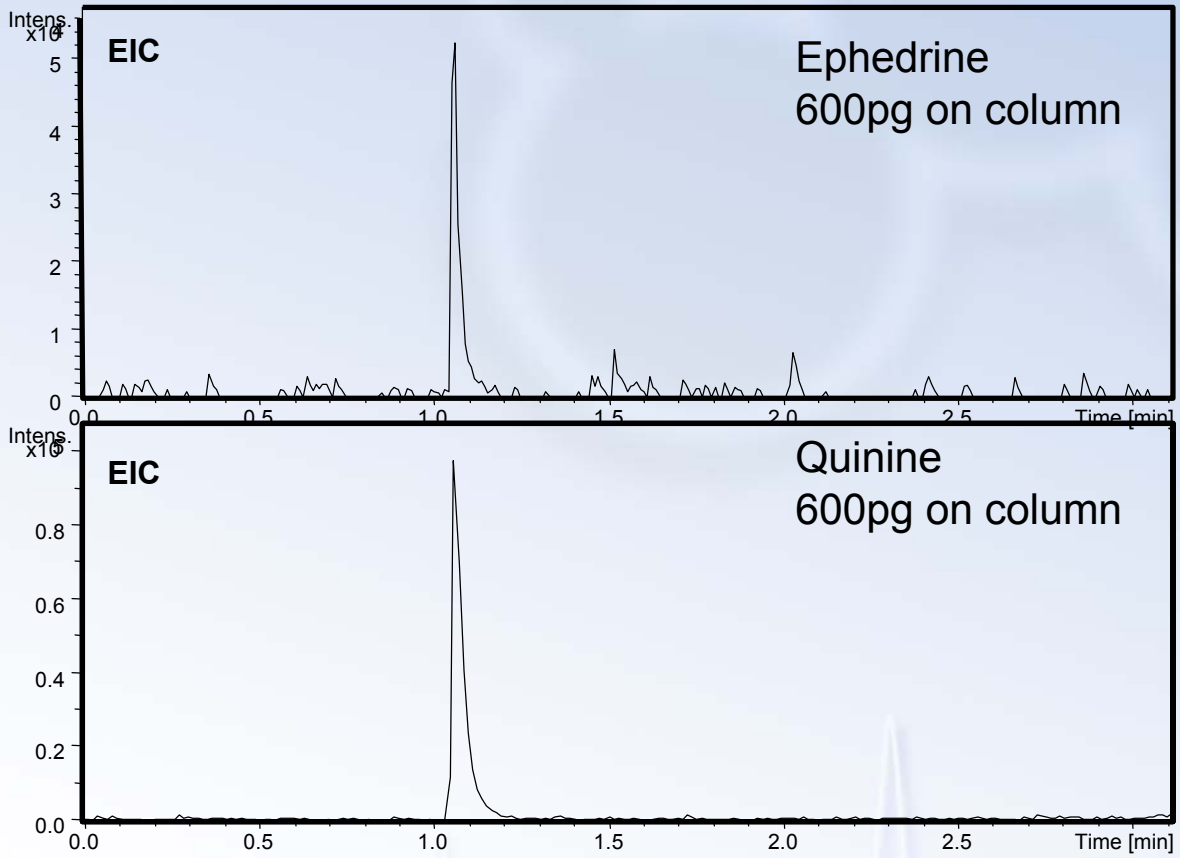
Range of Drug Compounds Investigated

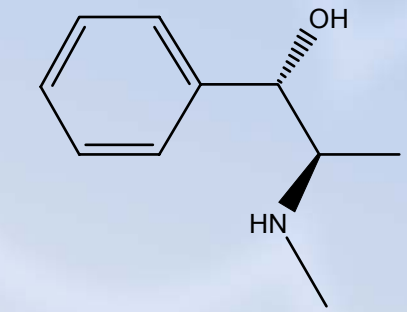
Lyophilized porcine plasma diluted 1:1 with water was spiked with the appropriate concentration of each analyte. Standards were obtained from Sigma (St. Louis, MO, USA).

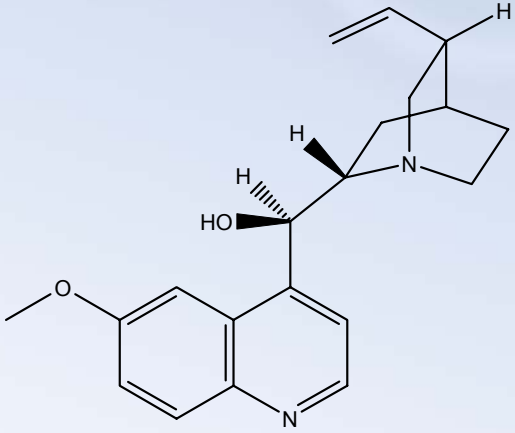
Standards:	LogP	MW
Acyclovir	-1.56	225.2
Atenolol	0.16	266.3
Ephedrine	1.13	165.2
Pindolol	1.75	248.3
Clozapine	3.23	326.8
Quinine	3.44	324.4
Fluoxetine	4.05	309.3



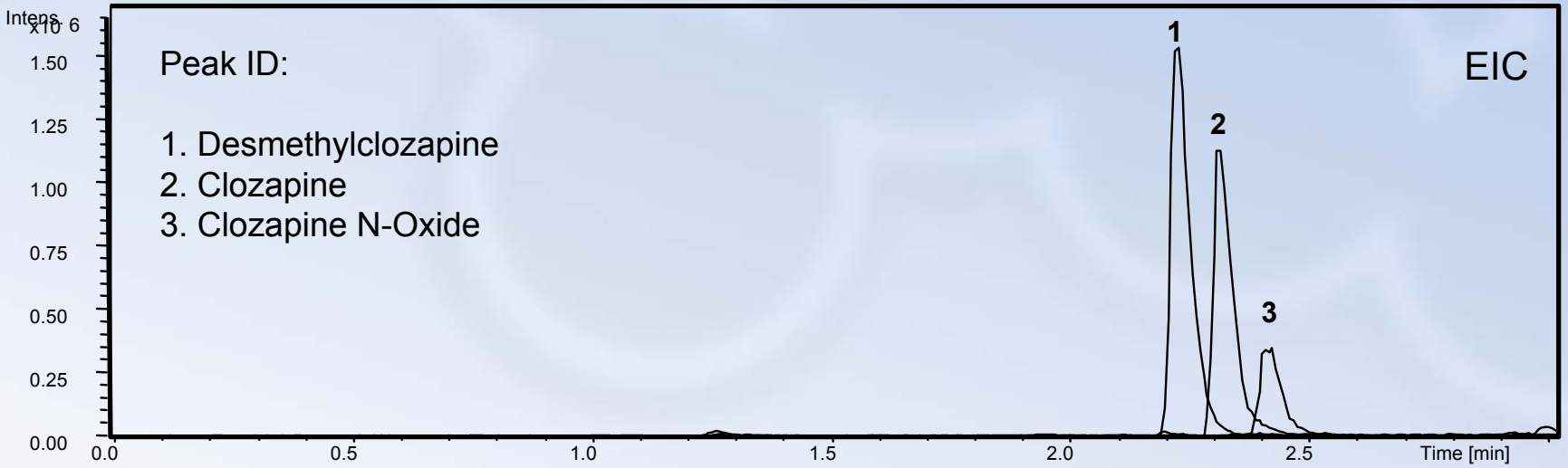
On-line SPE of Ephedrine and Quinine

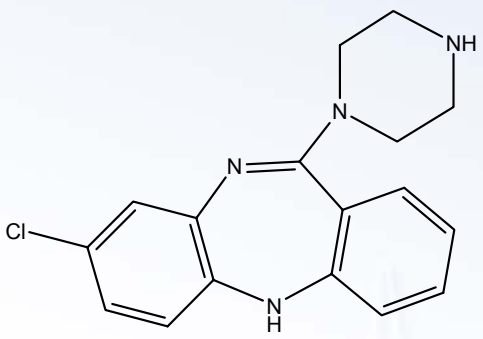




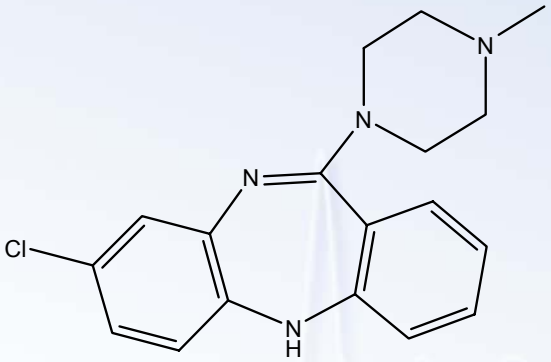


The On-line SPE of Clozapine and Metabolites

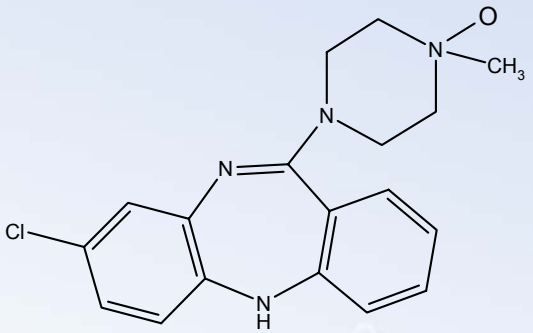




Desmethylclozapine



Clozapine



Clozapine N-Oxide

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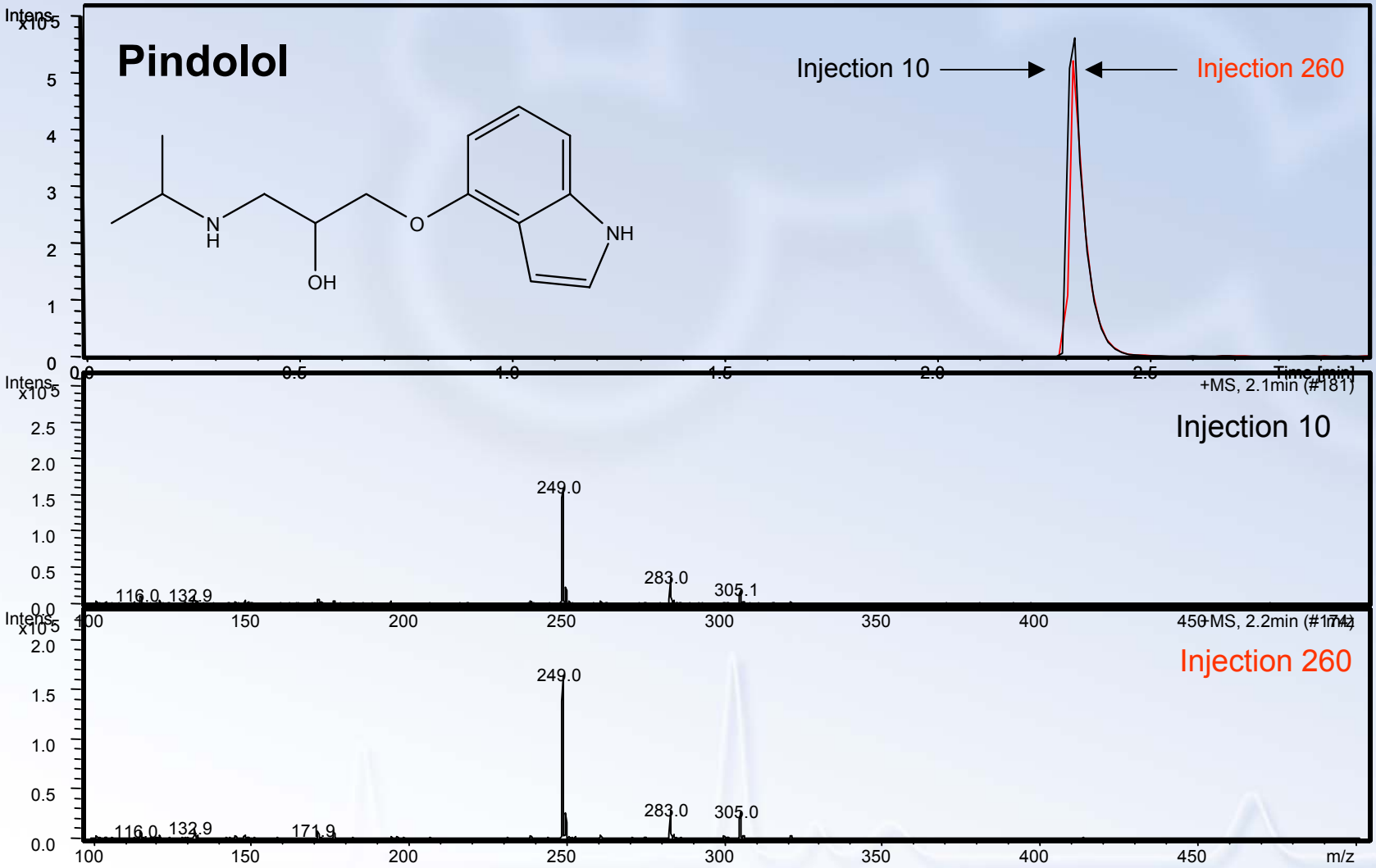
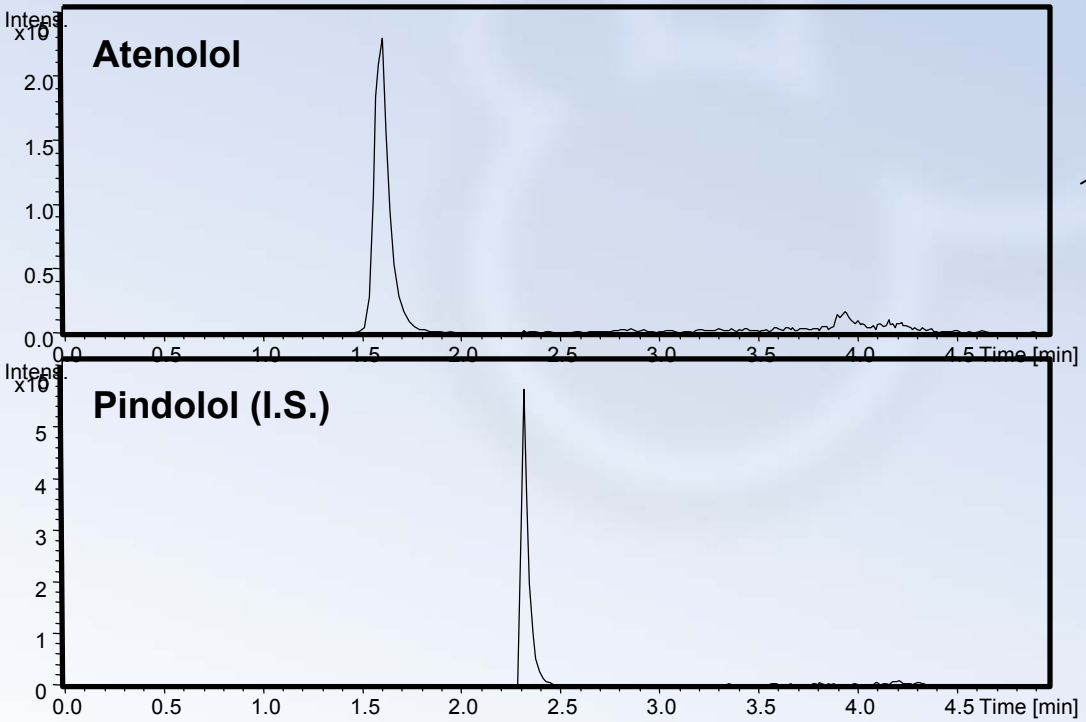
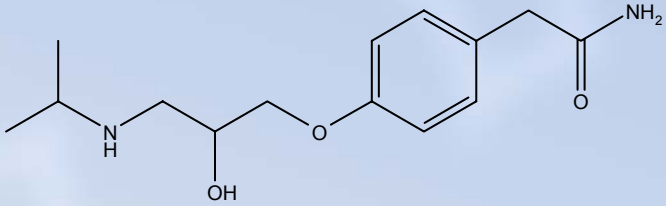
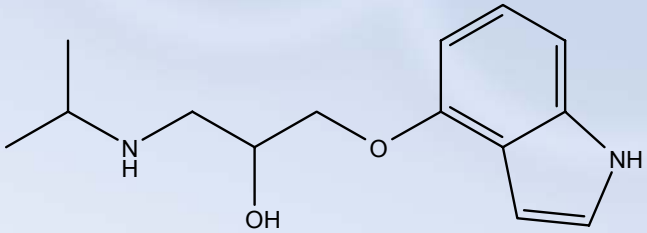
Greater than 200 injections in Plasma



Recovery of Atenolol in Plasma (Reversed Phase)







Concentration	Recovery %	RSD%
Atenolol 4 ng/mL (200pg on column)	**90.8**	**(1.4)**
Atenolol 20 ng/mL (2ng on column)	**98.5**	**(2.6)**

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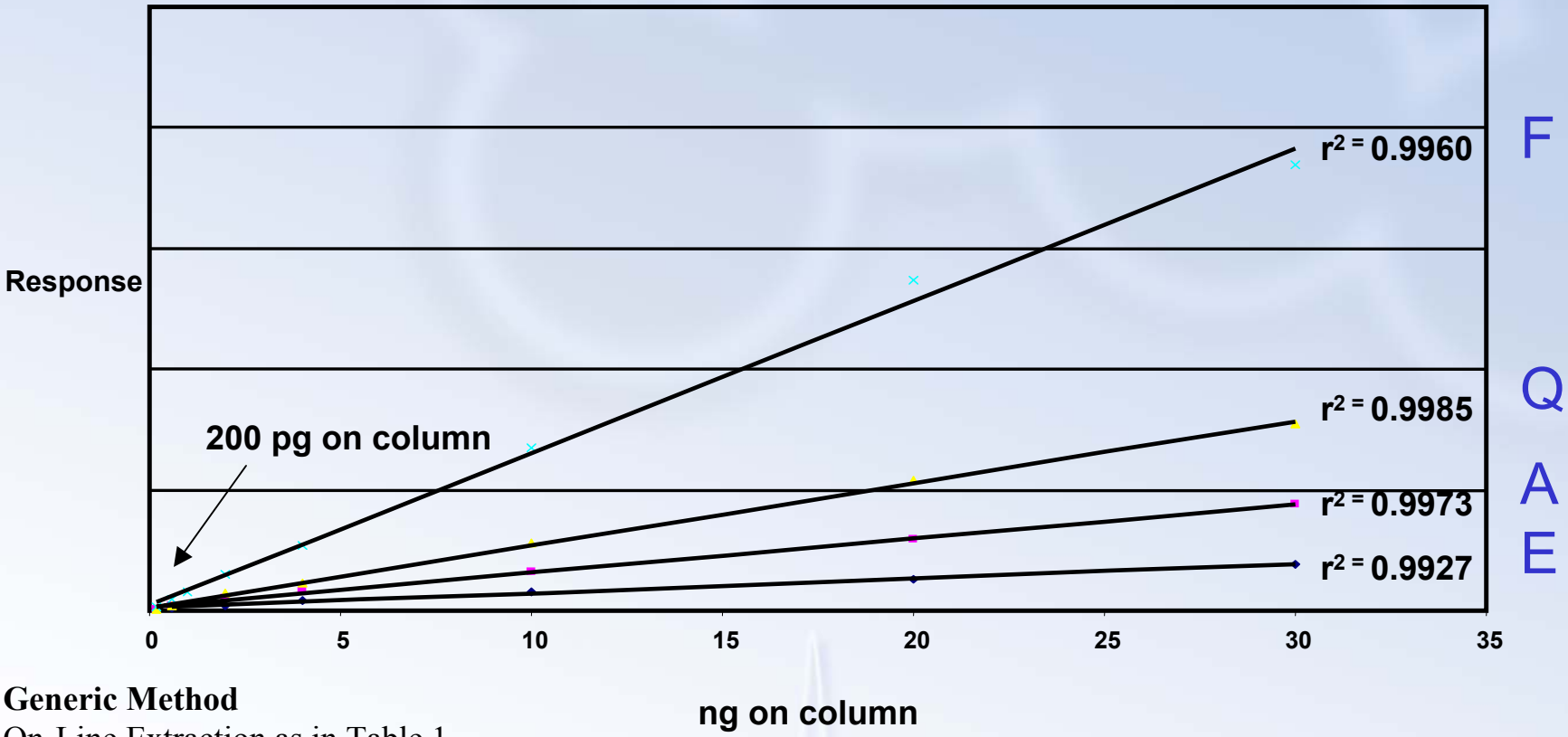
Linearity of Four Compounds Extracted in Plasma Using Strata™-X On-Line SPE



Generic Method

On-Line Extraction as in Table 1

Analytical Column: Synergi Hydro-RP 4µ (**A**tenolol and **E**phedrine)

Luna C18(2) 5µ 50x4.6mm (**Q**uinine and **F**luoxetine)

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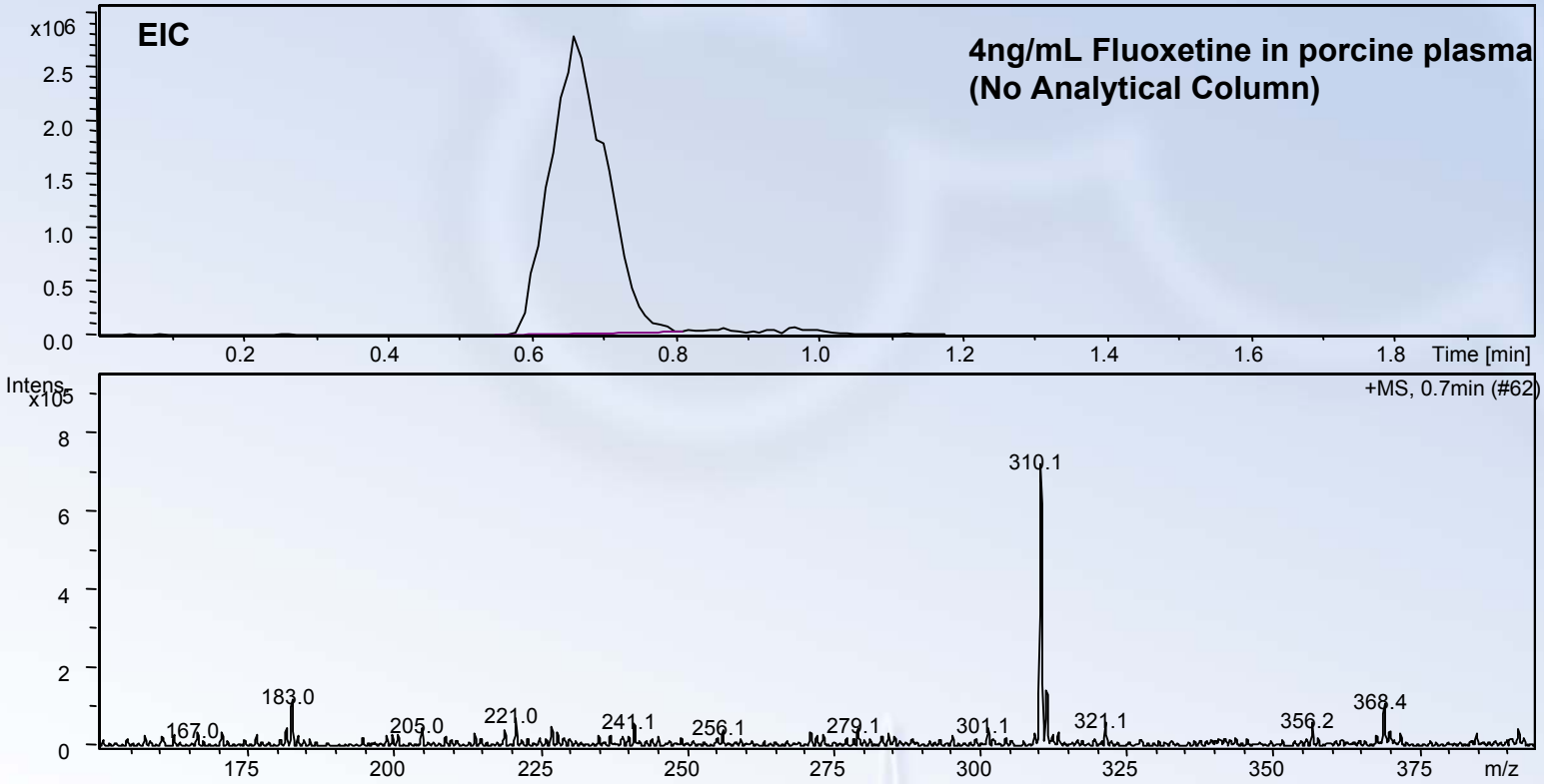
Even Shorter Cycle Times Without Analytical Column



- No analytical column, cycle time equals 1.2 minutes
- With analytical column, cycle time equals 3 minutes

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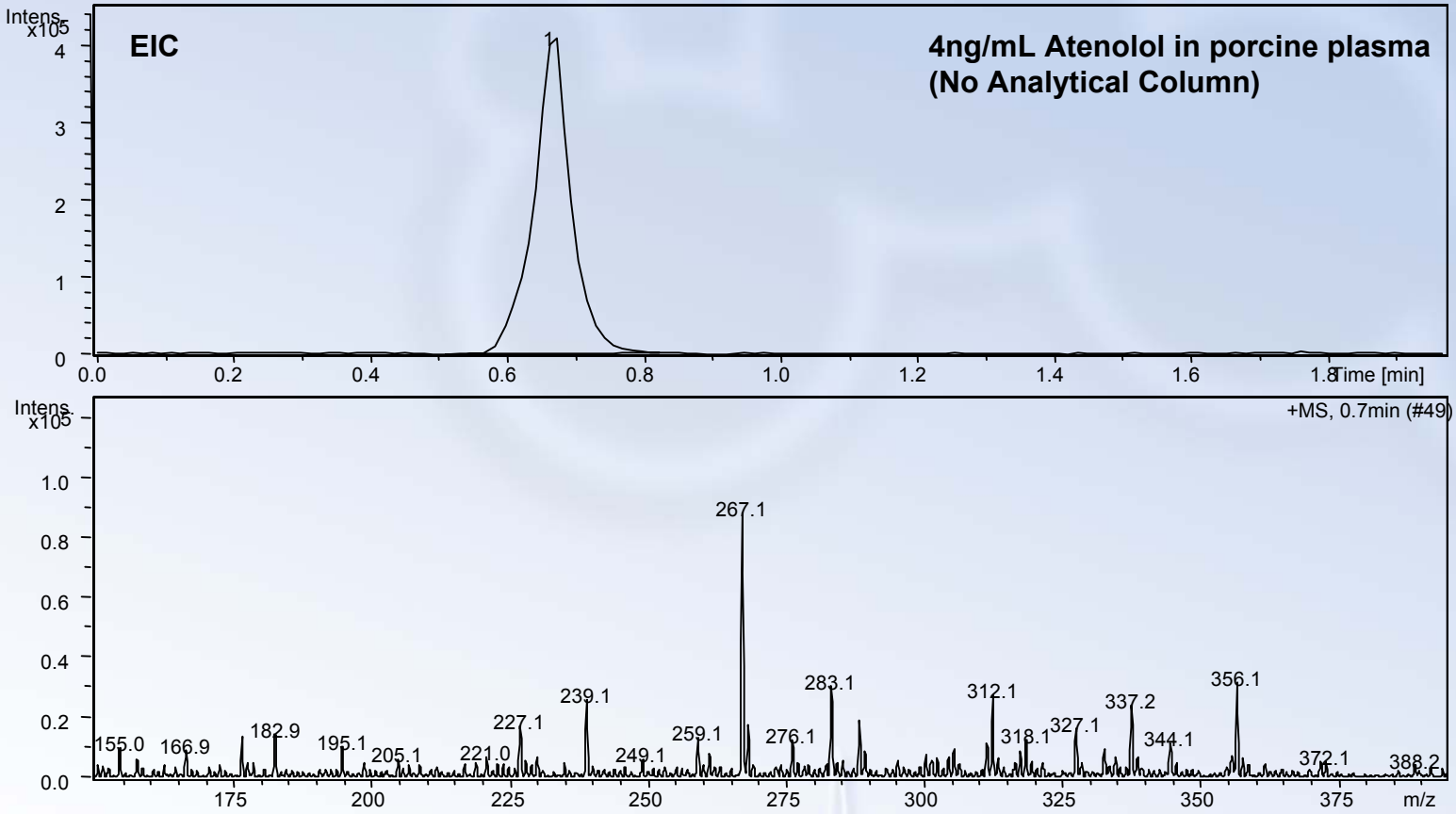
Even Shorter Cycle Times Without Analytical Column



- No analytical column, cycle time equals 1.2 minutes
- With analytical column, cycle time equals 3 minutes

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Discussion of Results

The data presented in this study **demonstrates the capability of on-line SPE using Strata™ -X On-line extraction cartridges**. This functionalized polymeric sorbent performs well for **both polar and hydrophobic drugs** in plasma. An example of the on-line SPE of drug substance (clozapine) and its metabolites is also presented to demonstrate the applicability of this technique for drug metabolism/pharmacokinetic studies (DMPK). Sample cleanliness, recovery and linearity over the wide concentration range typically investigated during drug discovery/development had been demonstrated with reliability. While recoveries obtained through on-line SPE match those obtained with off-line SPE protocols, the on-line technique offers much faster total sample prep and analysis time. The entire on-line process is automated, and the same on-line extraction cartridge can be used for numerous sample injections. This simplified protocol allows for significant cost savings in consumables (off-line SPE cartridge), solvents and waste. **Furthermore, on-line SPE columns can be used either in a stand alone fashion (for less demanding separations) or in tandem with an analytical column, a choice not available with off-line SPE.** In addition, on-line SPE can be successfully coupled to analysis in HILIC conditions when analyzing highly polar compounds that may be difficult to retain. Detection limits of 100-200 picograms can be achieved **and higher sample loads compared to off-line SPE can be utilized without compromising cleanliness or linearity**.

Conclusion

- On-line Solid Phase Extraction (SPE) is an attractive alternate to off-line SPE for the rapid analysis of drugs in biological matrices
- On-line SPE does not compromise cleanliness, recovery yields or linearity over a wide concentration range compared to off-line SPE, while it significantly decreases overall sample prep, analysis time and cost per sample.
- On-line SPE offers a choice of two modes of operation; (1) use of a stand alone SPE cartridge or (2) employing the SPE cartridge in tandem with an analytical column for more challenging separations
- On-line SPE allows simultaneous extraction, concentration, and separation
- On-line SPE is suitable for the analysis of drug/metabolite combinations in biological matrices.
- On-line SPE, when used in tandem with an analytical column, performs using reversed phase or HILIC conditions.

References

• J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb; Optimization and routine use of generic ultra-high flow-rate liquid chromatography with mass spectrometric detection for the direct on-line analysis of pharmaceuticals in plasma. J. Chromatography A, 828 (1998) 199-207.

• R.C. King, C.Miller-Stein, D.J. Magiera, J. Brann; Description and validation of a staggered parallel high performance liquid chromatography system for good laboratory practice level quantitative analysis by liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrometry, 2002, 16, 43-52.

• J.L. Herman; Generic method for on-line extraction of drug substances in the presence of biological matrices using turbulent flow chromatography. Rapid Commun. Mass Spectrometry, 2002, 16, 421-426.

• S.D.Garbis, A.Melse-Boonstra, C.E. West, R.B. van Breemen; Determination of folates in human plasma using hydrophilic interaction chromatography-tandem mass spectrometry. Anal. Chem. 2001, 73, 5358-5364.