Fast, Sensitive Drug Analysis Using Capillary HPLC/MS

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Overview

- A screening method for samples containing small drug molecules was developed using short capillary columns with fast flow rates and steep gradients
- A trapping column with a switching valve allowed for the injection of large sample volumes and on-line sample clean up
- Analysis times ranged from 5 to 7 minutes depending on system configuration
- The screening method was evaluated in terms of
  - Volume loading
  - Linearity
  - Sensitivity
  - Carryover

Introduction

- Capillary LC systems are gaining popularity due to their ability to increase sensitivity and handle small sample volumes
- Little emphasis has been given to capillary LC in bioanalysis
  - Concern given primarily to turnaround time and sample throughput
- As pharmaceutical development is increasingly directed at higher potency drugs, sensitivity is becoming more important in bioanalysis
- Through the use of high flow rates and steep gradients, capillary LC can be coupled with mass spectrometry to provide sensitive analyses

General Method Parameters

LC Conditions

Chromatographic Parameters

Instrument: Waters® CapLC™ System
Trapping Column: 0.18x30 mm Oasis® HLB, 25 µm
Analytical Column: 0.32x50 mm XTerra™MS C18, 5 µm
Mobile Phase A: 0.1% Formic Acid in MilliQ Water
Mobile Phase B: 0.07% Formic Acid in MeCN
Loading Solvent: 0.1% Formic Acid in MilliQ Water
Loading Flow Rate: 40 µl/min
Gradient Flow Rate: 30 µl/min
Gradient Slope: 5 to 95% B in 3 min

Direct Injection Method

3 min gradient
1 min hold at 95% B
1 min re-equilibrate at 5% B
5 min total run time

Trapping Method

Load sample for 2 min
3 min gradient
1min hold at 95% B
1 min re-equilibrate at 5% B
7 min total run time

Sample Information

Standards: 5 drugs + internal standard (IS) in H_2O
Plasma: 1:1 dilution with standards and IS in H_2O
Injection Volume: 1 to 20 µl
IS concentration adjusted to yield 50 pg on column

MS Conditions

MS Acquisition Parameters

Instrument: Waters Micromass ZMD
120 µm Standard Capillary
Positive Electrospray
Centroid Data
SIR Mode
Dwell Time: 20 ms
Interchannel Delay Time: 10 ms

MS Tune Page Settings

Capillary: 3.2 kV
Extractor: 5 V
Ion Energy: 0.4 V
LM Resolution: 15.0
HM Resolution: 15.0
Source Block Temperature: 100°C
Desolvation Temperature: 120°C
Desolvation Gas: 740 l/hr
Cone Gas: 100 l/hr
Cone: 28 V
RF Lens: 0.1 V
Multiplier: 800

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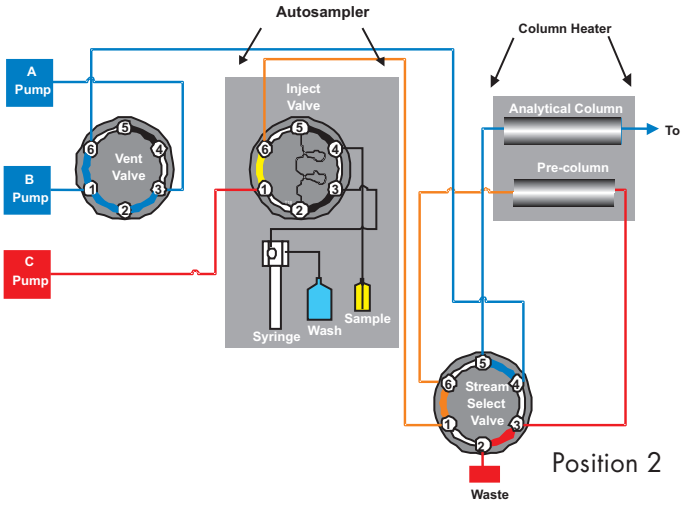
Method Development

Sample Trapping and On-Line Extraction Configuration

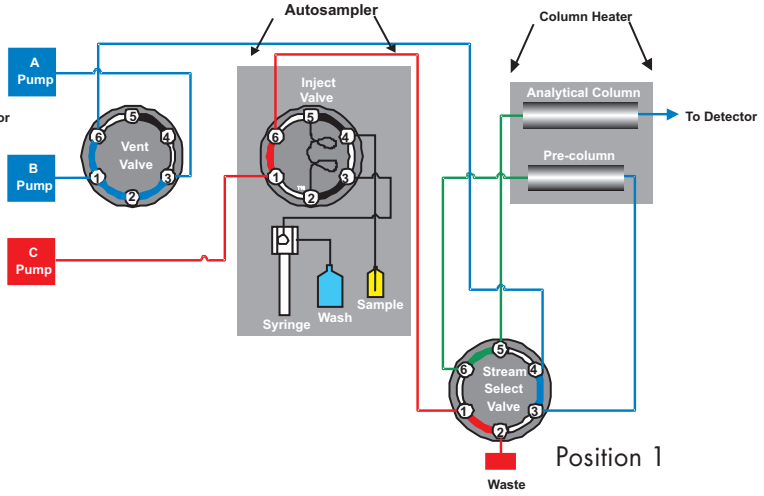
Sample Loading

- Sample was loaded onto the precolumn at a high flow rate using Pump C for 2 minutes
- Using an integrated 6 port, 2 position valve, the sample was trapped on the precolumn
  - Loading solvent was directed to waste after the precolumn
- By selecting the appropriate stationary phase, the precolumn can be used for a variety of purposes
  - Sample focusing
    - Large sample volumes can be concentrated on the precolumn to minimize volume overload of the analytical column
  - On-line sample cleanup
    - Samples can be loaded onto the precolumn and rinsed with the loading solvent to remove complex matrices such as plasma

Sample Loading



Sample Separation



Sample Separation

- Once the sample was loaded, the position of the stream select valve was changed and the sample was eluted from the precolumn into the analytical column
  - The gradient was formed by Pumps A and B
- The precolumn is backflushed in the configuration shown here
  - Backflushing allows for the focusing of sample bands

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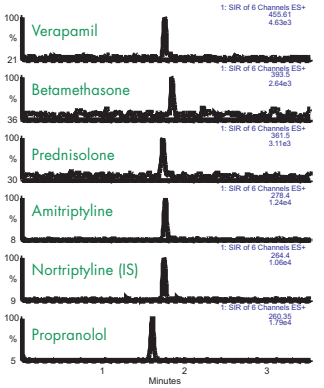
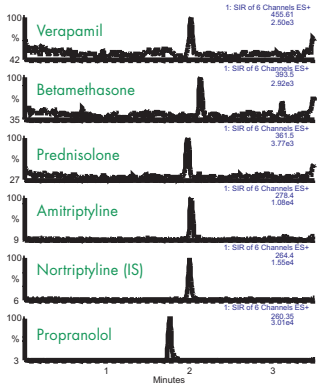
Loading

Direct Injection

1 µl Injection of Standard Mixture
50 pg On Column

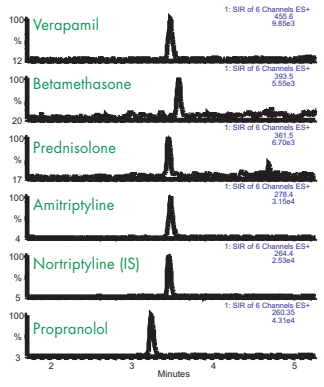


20 µl Injection of Standard Mixture
50 pg On Column

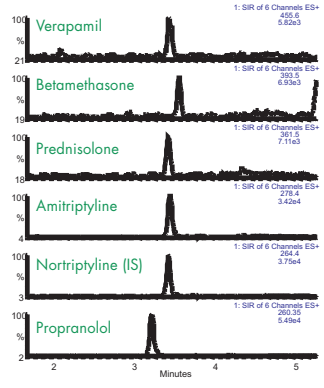


Trapping

1 µl Injection of Standard Mixture
50 pg On Column



20 µl Injection of Standard Mixture
50 pg On Column

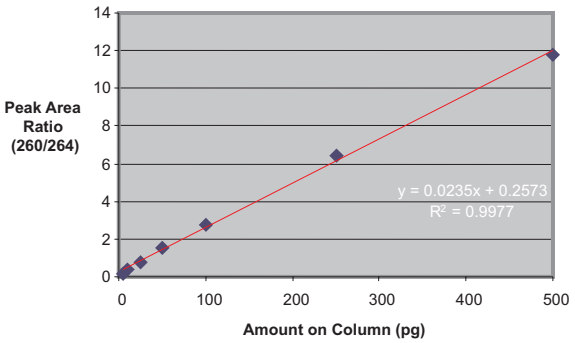


With these particular molecules, good separation was maintained when large volumes of the drug mixture were directly injected. Although trapping these molecules yielded some band broadening, the trapping configuration afforded the opportunity for on-line sample clean up.

Linearity

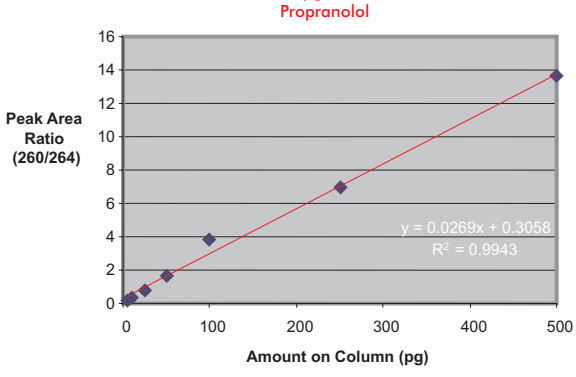
Trapping

1 µl Injections of Standard Mixture
5 to 500 pg On Column
Propranolol



Trapping

20 µl Injections of Standard Mixture
5 to 500 pg On Column
Propranolol



Good linearity with an example molecule, propranolol, was achieved when both small and large injection volumes were used.

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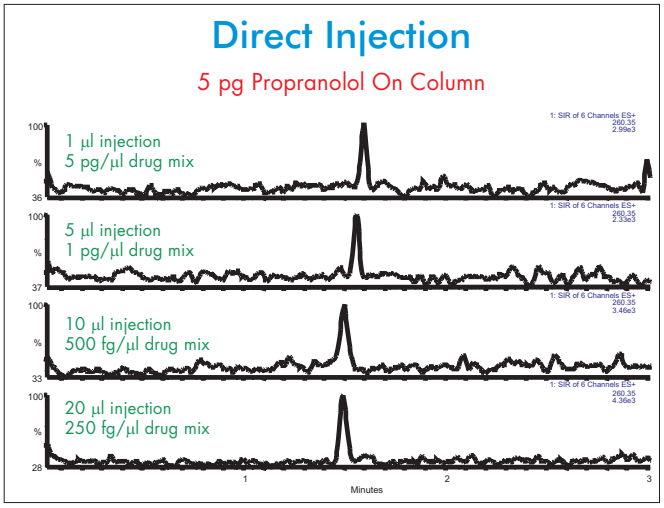
Sensitivity

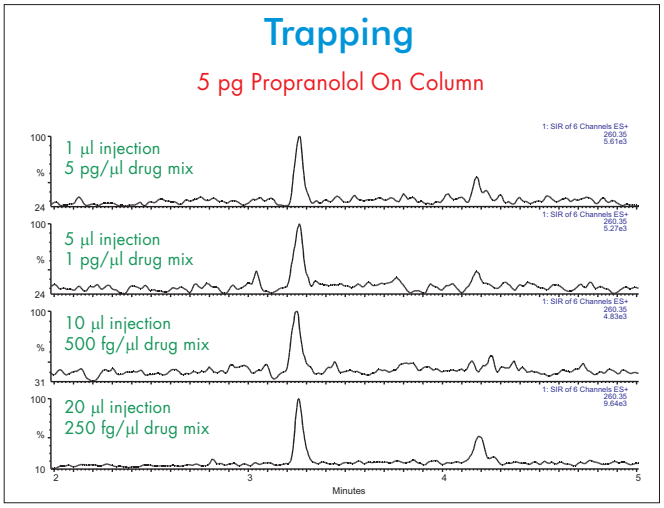
Direct Injection

5 pg Propranolol On Column



Trapping

5 pg Propranolol On Column



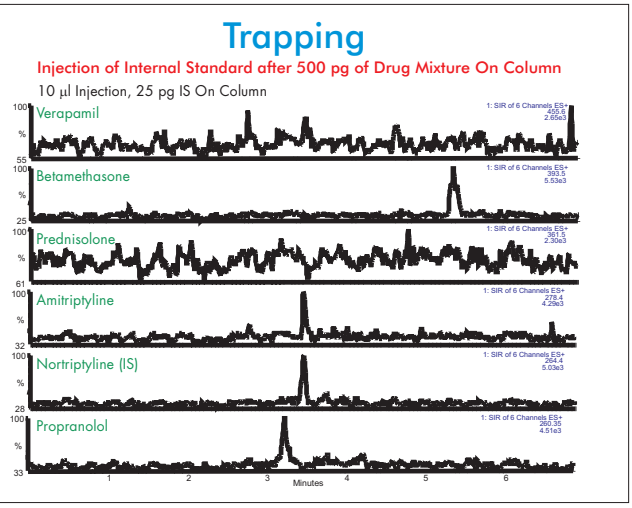
Similar sensitivities were attained for propranolol using direct injection and trapping methods.

Carryover

Trapping

Injection of Internal Standard after 500 pg of Drug Mixture On Column

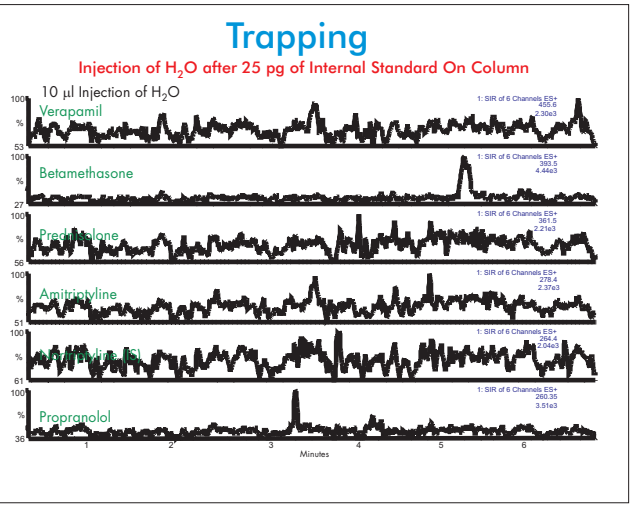
10 µl Injection, 25 pg IS On Column



Trapping

Injection of H_2O after 25 pg of Internal Standard On Column

10 µl Injection of H_2O



After analyses with 500 pg of each mixture component on column, an injection containing only the internal standard was made followed by an injection of H_2O. A small amount of carryover for amitriptyline and propranolol was evident in the injection containing the internal standard. However, in the next injection consisting solely of H_2O, the carryover was dramatically reduced.

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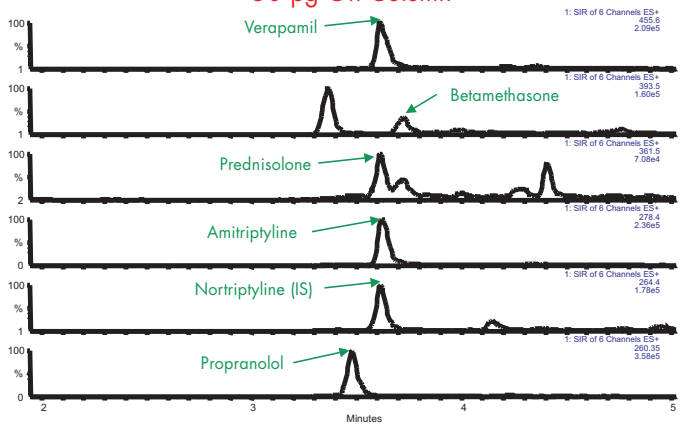
Trapping with Plasma Sample

1 µl Injection of Plasma Spiked with Standards
50 pg On Column



Plasma, diluted 1:1 with standards and IS in H_2O, was loaded onto a trapping column for on-line sample clean up. After rinsing the proteins from the sample on the trapping column, the drug molecules were eluted from the trapping column and separated. Although some peak broadening was seen, good signal to noise was achieved with 50 pg of each sample molecule on column (25 pg of IS).

In future work, we anticipate the ability to detect 10 to 25 pg of each compound on column. By increasing the injection volume, the concentration of sample should reduce to 1 to 2.5 pg/µl. Molecules for which this method was optimized, such as amitriptyline and propranolol appear likely to yield sub pg/µl detection.

It should be noted that there was no pretreatment, such as acidification, of the sample to minimize any protein-drug interaction. Also, the generic method shown here could be modified to optimize the loading and recovery of categorized drugs (i.e.. acids, bases, and neutrals).

Conclusions

- Small drug molecules can be analyzed using a fast capillary LC/MS method with a 5 to 7 minute run time
  - Sensitivity down to 5 pg on column for certain molecules
- Dilute samples or those in complex matrices can be analyzed by incorporating a column switching routine in the method
  - Method is completely automated
- On-line clean up of a diluted plasma sample can be performed with good signal to noise

Acknowledgements

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