

# **Application of SFC in Direct Assay of Aqueous Pharmaceutical Formulations**

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# Introduction

## SFC vs. HPLC :

- Higher speed of separation
- Higher efficiency
- Consumption of less mobile phase
- Cheaper and safer “CO<sub>2</sub>”
- “Green Chemistry”

## Chiral SFC in Pharmaceutical Industry:

- Rapidly replacing chiral HPLC as standard screening and automated method development technique
- Other major pharmaceutical and biotech. companies  
Pfizer, GSK, Novartis, Lilly, Merck, BMS, P&G, Amgen

## Pharmaceutical Formulation Assays by Chiral SFC :

- Relatively recent application area
- Primarily solid dosage forms (>95%) – Tablets, capsules
  - Dosage forms dissolved in methanol, filtered, assayed
- Solution dosage forms (<5%) – Suspensions, emulsions, solutions
  - Sample pretreatment : organic extraction from aqueous matrices
  - Different mobile phase : Isopropanol vs. methanol
  - Mobile phase additives : 1 mM citric acid

## **Direct Assay of Aqueous Formulations by SFC :**

- Extremely limited literature reports (5) due to :
  - Freezing of aqueous solutions
  - Precipitation of samples
  - Poor chromatography

## **Rationale for SFC Feasibility Study by AZ Wilmington ED :**

- Investigate the possibility of direct assay as a challenging R&T initiative
  - No sample treatment- inject formulation as it is- innovative
  - Higher sample throughput- increased productivity in Early Development (ED)
  - Exploit the least utilized separation technology in ED/US PARD
  - Demonstrate the suitability of this technique

## **Selection of Model Analyte for Feasibility Study :**

- Chiral in nature
- Both enantiomers or a racemic mixture available for reference and method development
- Selected from highly visible AZ projects of various TAs
- Compounds requiring a significant formulation and analytical support
- Preferably CDs for AZ (thus demonstrating impact of this technique in R&D)
- Compounds of diverse chemical nature (versatility of the technique)

## **Selection of SFC Analytical Column for MD :**

- Chiralpak AD > Chiralcel OD > Chiralcel OJ > Chiralpak AS  
(> 90% of pharmaceuticals analyzed)
- Chiralpak AD-H selected as a starting point

## Part I : Direct Aqueous Assay of a Basic AZ compound : AZM

### Materials :

- AZM- R and AZM-S (initial optical purity 99%)
- HPLC grade methanol and 200 proof ethanol- mobile phase
- Isopropylamine and dimethylethylamine – mobile phase additives
- SFC grade CO<sub>2</sub> - Grade 5.0
- Preformulation vehicles
  - 0.1 M lactic acid (pH 3.0)
  - 0.05 M potassium phosphate monobasic buffer (pH 7.0)
  - Sodium borate buffer (pH 9.5)

## **Methodology :**

### **Experimental Procedure :**

- Racemic mixture of AZM-R and AZM-S in 0.1 M lactic acid formulation vehicle

(0.05 mg/mL)- SFC MD

-For evaluation of direct assay of 100% aqueous solutions of AZM-R

(1). Approx 1 mg/mL of AZM-R in 1 mL of 0.1 M lactic acid (pH 3.0)

(2). Approx 1 mg/mL of AZM-R in 1 mL of 0.05 M potassium phosphate monobasic buffer (pH 7.0)

(3). Approx 1 mg/mL of AZM-R in 1 mL of 40g/L sodium borate buffer (pH 9.5).

AZM was completely soluble in pH 3.0 solution.

At pH 7.0, and 9.5, the compound was sparingly soluble and the saturated solutions (2) and (3) were filtered. The exact concentrations of these filtered solutions at pH 7 and pH 9.5 were, therefore, not known. This was acceptable for the qualitative purpose of this study.

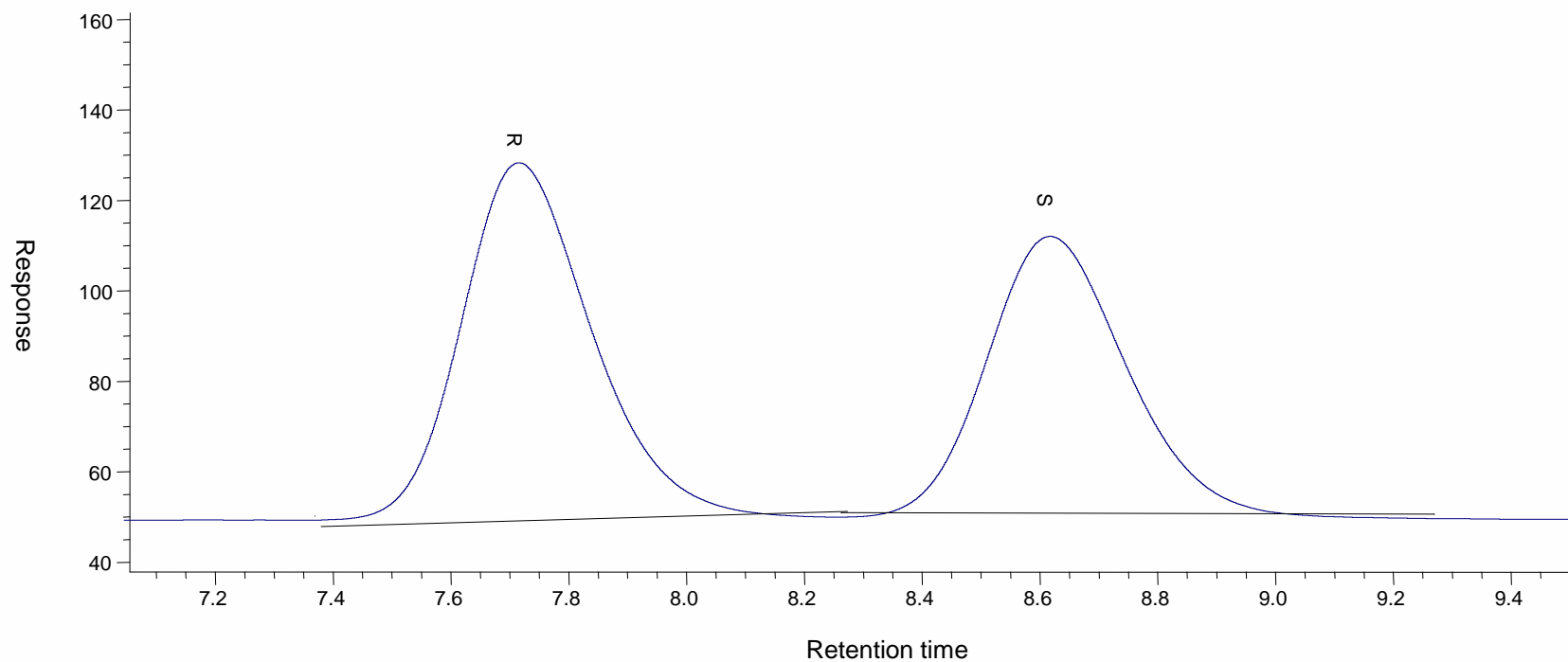
## Methodology :

### SFC Chromatographic System

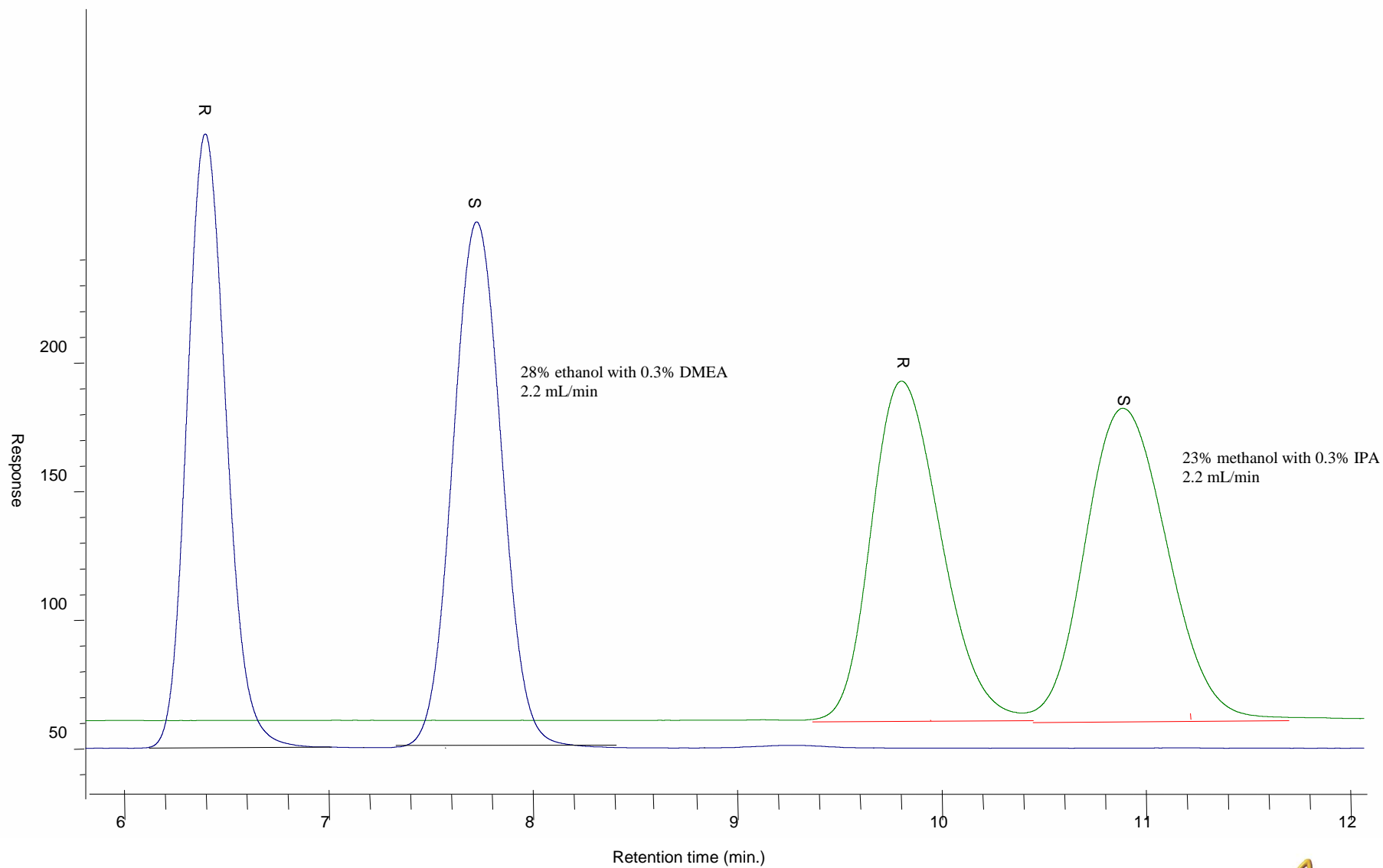
Component	Description
SFC system	Berger
Autosampler	Berger ALS model 719
Sample Injection Volume	40 $\mu$ L
Detector	HP 1050
Detection wavelength	244 nm
Analytical Column	Chiralpak AD-H, 5 $\mu$ , 4.6X 250 mm, Chiral Technologies
Mobile Phase 1	23% Methanol with 0.3% v/v IPA /77% CO <sub>2</sub>
Mobile Phase 2	28% Ethanol with 0.3% v/v DMEA/72% CO <sub>2</sub>
Flow Rate	2.2 mL/min
Nozzle Temperature	60°C
Oven Temperature	40°C
Data acquisition	HP Chemstation and Thermoelectron Atlas
Modifer Switching valve	Six position Valco

## Results :

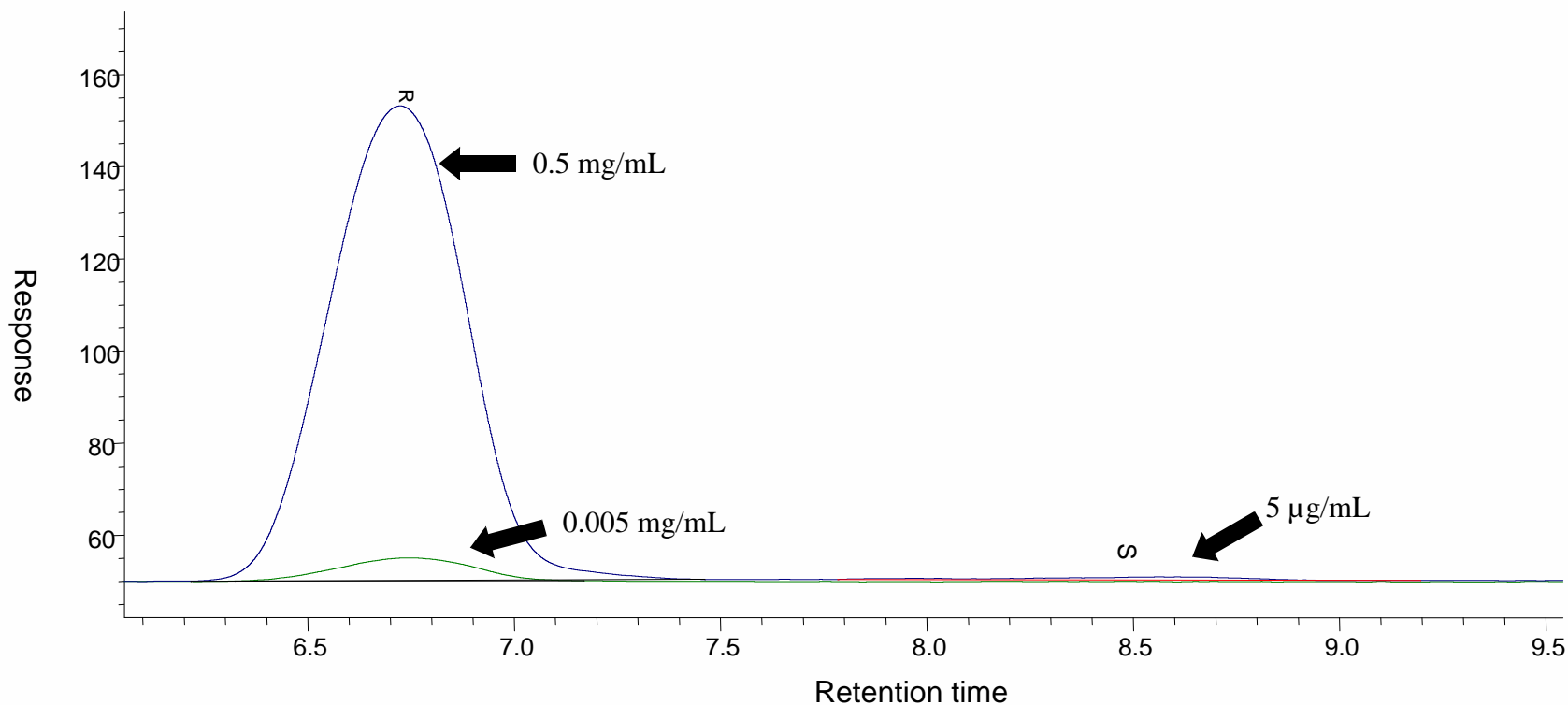
Chiral SFC of 100% Aqueous Solution (0.1 M lactic acid, pH 3.0) of a Prepared Racemic Mixture of R and S enantiomers of AZM



# SFC method development for 100% aqueous formulation assay :



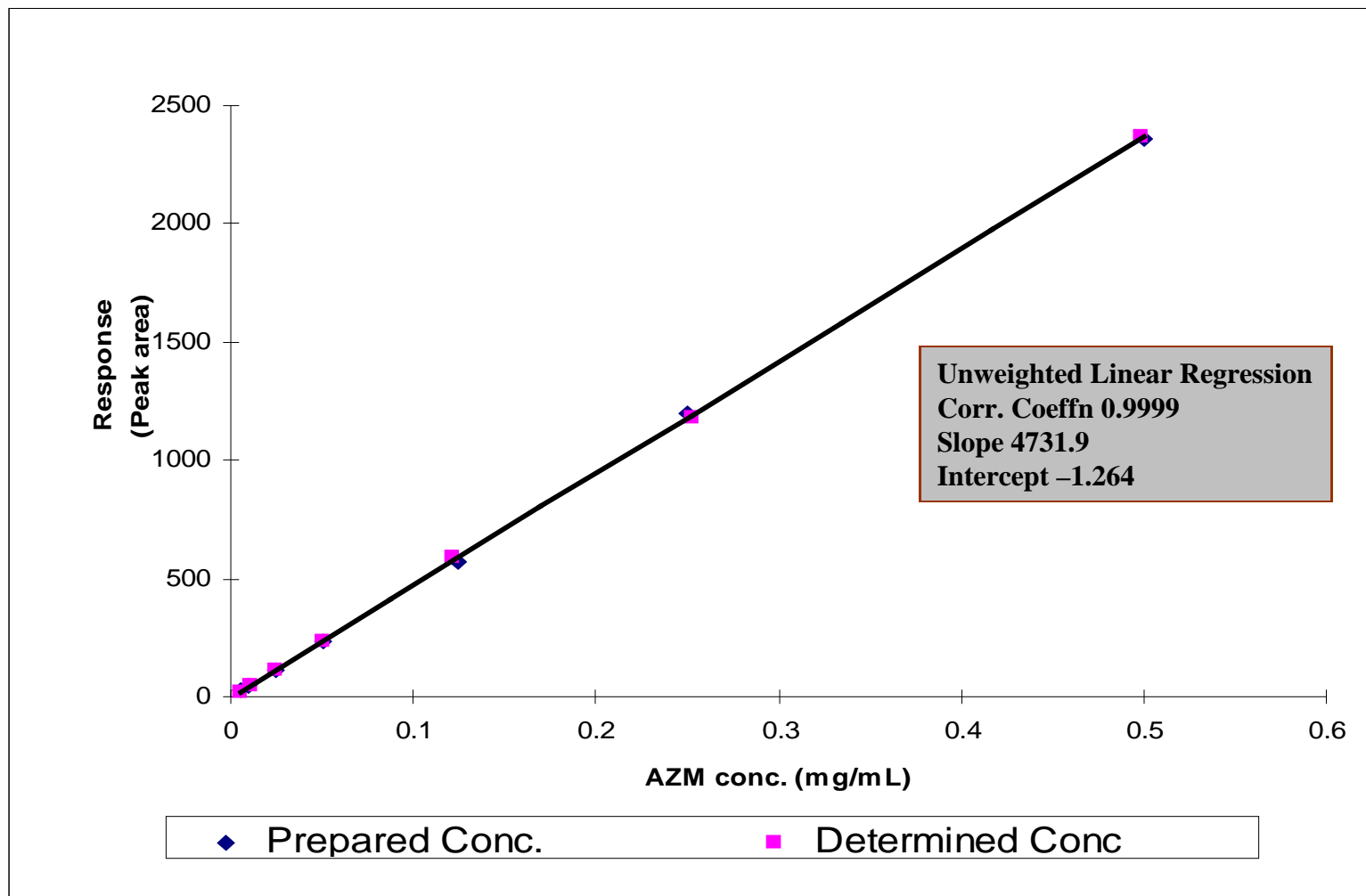
## Direct assay of 100% aq. formulation (0.1 M Lactic acid, pH 3.0) of AZM by Chiral SFC :



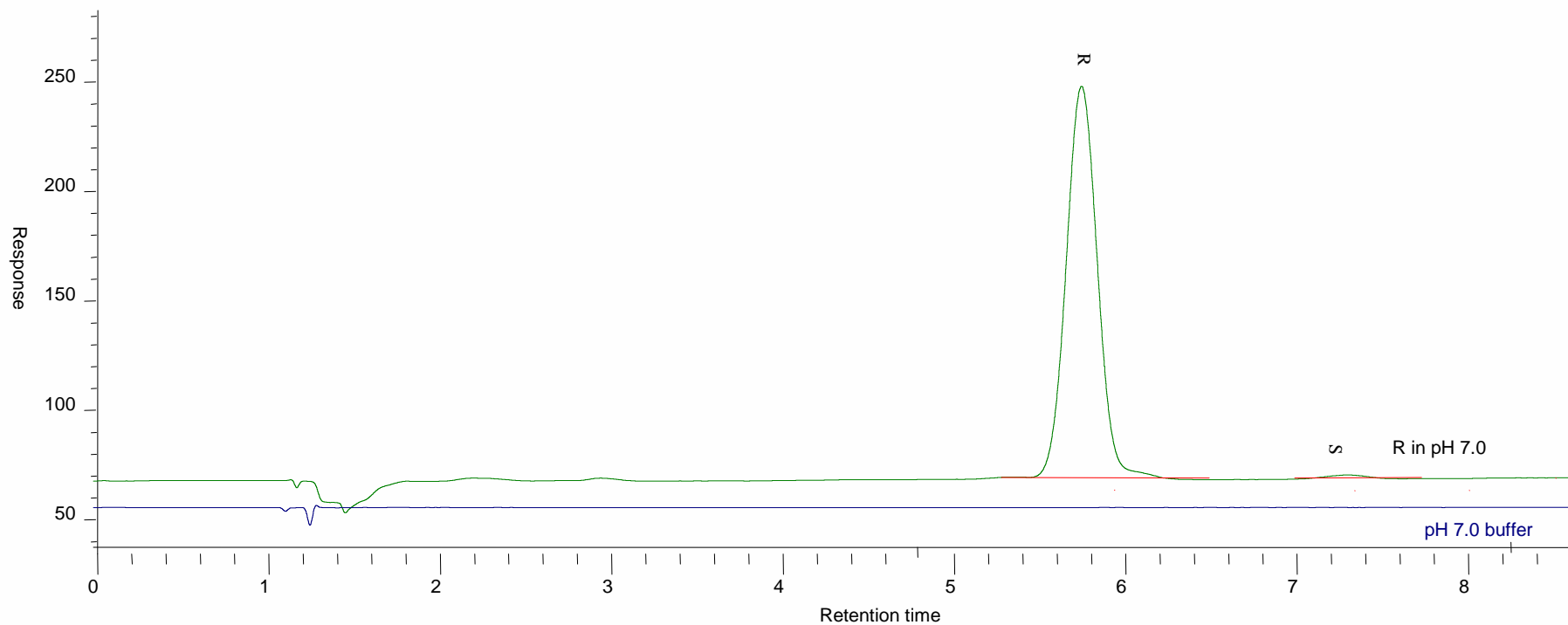
Initial optical purity of AZM-R ~99%

The assay could detect ~5 ppm of the chiral impurity S in the original lot of AZM-R even in aq solution

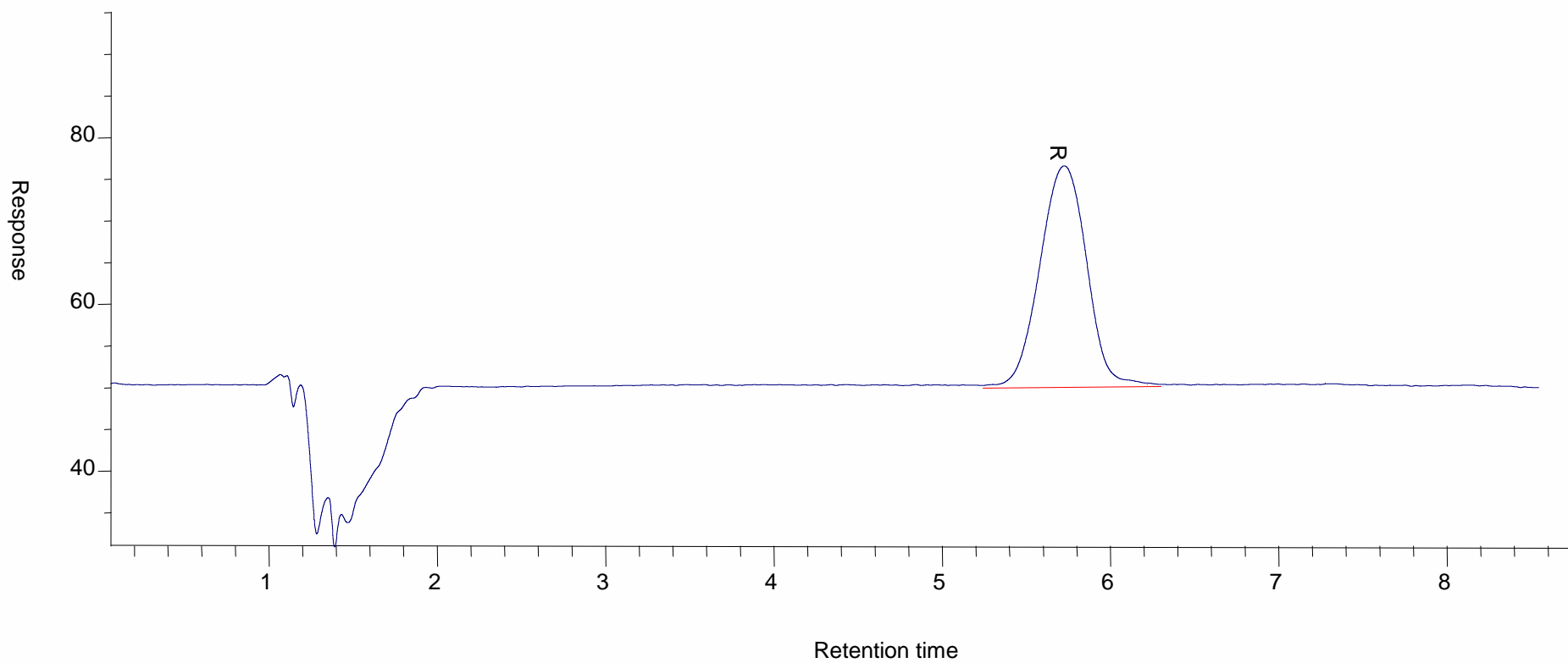
## Linearity of 100% Aqueous Formulation Assay by Chiral SFC :



# Typical Chromatogram of direct assay of 0.5 mg/mL AZM in pH 7.0 buffer, by SFC :



**Typical Chromatogram of direct assay of 0.5 mg/mL AZM in pH 9.5 buffer,  
by SFC :**



## Summary of Results from AZM Direct Assay by SFC :

- Selective method with short analysis time, even under not completely optimized condition
- Analyzed 100% aq formulations at pH 3.0, 7.0, 9.5 consistently
- Sensitive assay, could detect <1% S impurity in aq. formulation pH 3.0
- No sample pretreatment, no special mobile phase or additive
- Linear response between 0.005 and 0.5 mg/mL,  $r = 0.9999$
- DL and QL 0.75  $\mu\text{g/mL}$  and 2.5  $\mu\text{g/mL}$ - could be further improved
- Accurate ( $\pm 4\%$ ), and precise (1.3%) assay

## **Part II : Direct SFC Assay and Validation for a Free Acid AZ Compound (AZY)**

### **Purpose :**

- To further explore and extend the applicability for an acidic pharmaceutical compound
- To validate the analytical SFC method (FDA and ICH) to demonstrate that the direct assay technique is :
  - selective
  - accurate
  - precise
  - sensitive
  - linear
  - robust for daily application in formulation analysis

## Methodology :

### Materials

#### *Preformulation Vehicle -*

0.05 M meglumine was prepared in 5% dextrose-water vehicle and the final pH adjusted to 7.4 with 0.1 N HCl. This formulation vehicle was used for preparation of standards, QCs, and in other validation experiments

#### *Reference Solution -*

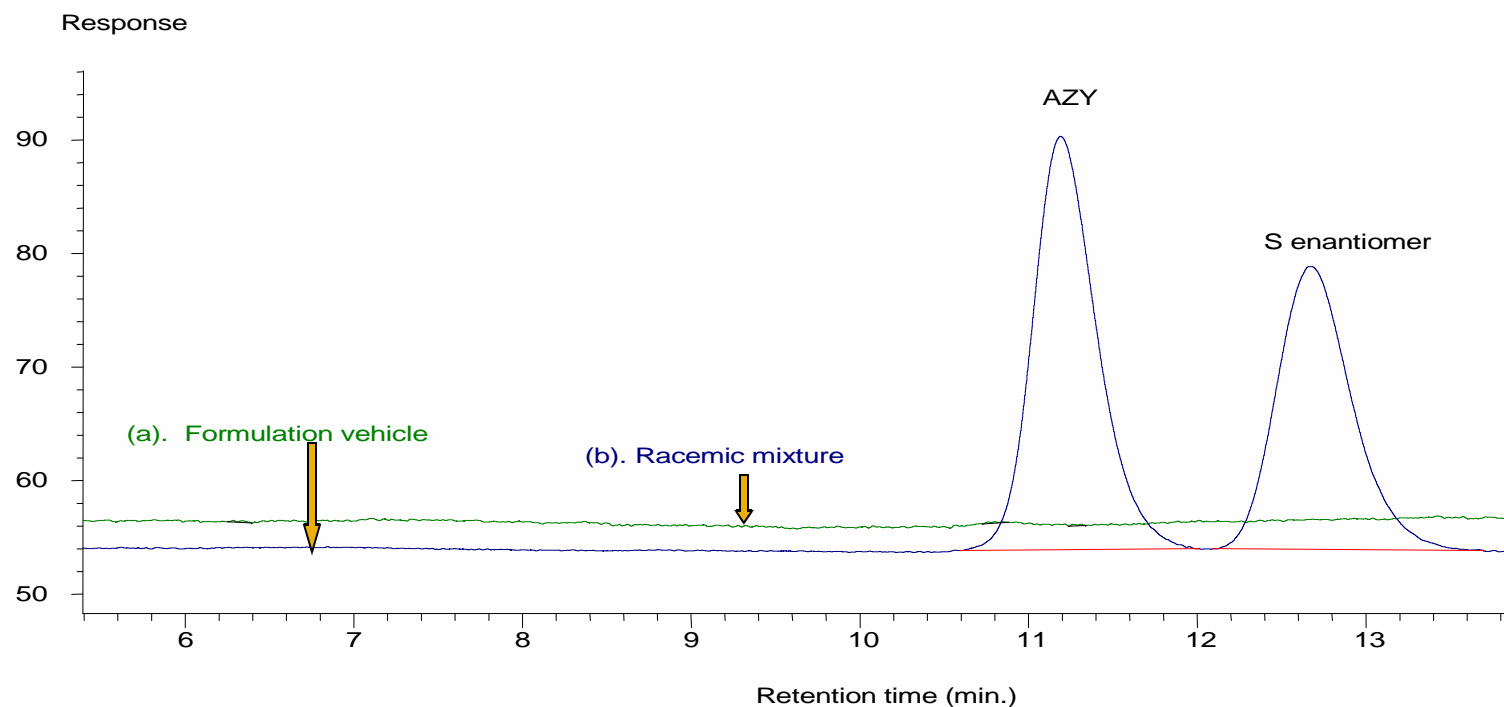
For method development purpose, a 0.05 mg/mL solution of the racemic mix of AZY and its enantiomer was prepared in 0.05 M meglumine. This solution was used as the SFC method development reference, to ensure baseline resolution of the enantiomers by SFC

#### *Mobile phase, additive, CO<sub>2</sub>*

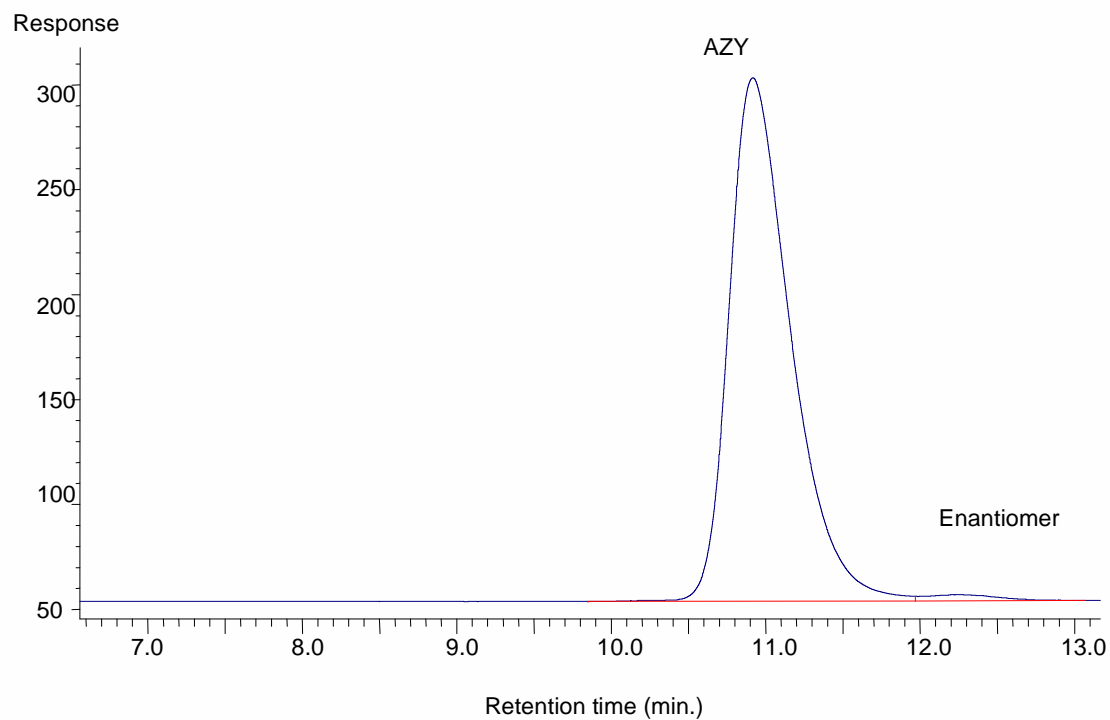
## Methodology : SFC Instrumentation

Component	Description
SFC system	Berger Analytix
Autosampler	Alcott Chromatography model 719
Sample injection volume	20 µl
Pump	Berger fluid control module chiral drug FCM 1100
Column Compartment	Berger Thermal Control Module TCM 2000
Modifier selection valve	6 port Valco model C22Z
Detector	Agilent PDA
Detection wavelength	290 nm
Analytical column	Chiralpak AD-H (Lot ADH0CE-FC016), 4.6X250 mm, 5 µ
Mobile phase	30% ethanol with 0.3% DMEA/70% CO <sub>2</sub>
Flow rate	3.0 ml/min
Oven temperature	40°C
Nozzle temperature	60°C
Back pressure	100 bar
Data acquisition	BI-SFC Chemstation 3.6.6 and Thermoelectron Atlas

## Results :



Demonstration of selectivity of the direct assay. Chromatogram (a) represents blank solvent vehicle (0.05 M meglumine, pH adjusted to 7.4). Chromatogram (b) represents the racemic mixture of AZY and its enantiomer in the same solvent, separated using 70% CO<sub>2</sub>/30% ethanol +0.3% DMEA, 3 ml/min, 40°C, 100 bar.



Chromatogram showing ~1.3% chiral enantiomeric impurity in 1 mg/ml of AZY formulated in meglumine formulation vehicle. The assay could reproducibly detect the minor impurity in all samples analyzed.

## Regression Analysis Parameters :

Day	Correlation Coefficient	Slope (mV.ml/mg)	Intercept	Std Error
1	0.9997	8849.05	-0.8630	65.70
2	0.9995	8330.15	-1.3147	78.15
3	0.9994	8824.21	-2.4831	89.43
4	0.9988	8580.46	-1.6604	115.52
Mean	0.9993	8645.97	-1.5803	87.20
Std dev	0.0003	242.93	0.6847	21.22
-95% CI	0.9983	8019.20	-3.3469	32.45
+95% CI	1.00035	9272.73	0.1863	141.95

## Analysis Repeatability :

Day	Prep'd conc. (mg/ml)	Mean det'd conc. (mg/ml)	% RE	Mean % RE	% RSD	Mean % RSD	Mean RT (min.)	% RSD	Mean % RSD
1	0.2525	0.2515	0.4	0.4	0.9	1.6	11.17	0.5	1.1
2	0.2750	0.2723	1.0		2.4		10.99	0.9	
3	0.2650	0.2623	1.0		1.6		10.96	1.4	
4	0.2550	0.2567	-0.7		1.6		10.82	1.6	

## Intra- and inter-day accuracy of AZY QCs for direct assay :

<b>QC nominal conc.(mg/ml)</b>	<b>Validation day</b>	<b>Intraday mean (n=3) % RE</b>	<b>Interday mean (n=4) % RE</b>
<b>0.0111</b>	<b>1</b>	<b>-8.8</b>	<b>-2.2</b>
	<b>2</b>	<b>-0.9</b>	
	<b>3</b>	<b>3.7</b>	
	<b>4</b>	<b>-3.0</b>	
<b>0.111</b>	<b>1</b>	<b>-4.5</b>	<b>-0.9</b>
	<b>2</b>	<b>0.8</b>	
	<b>3</b>	<b>0.3</b>	
	<b>4</b>	<b>-0.3</b>	
<b>1.11</b>	<b>1</b>	<b>-2.9</b>	<b>0.6</b>
	<b>2</b>	<b>4.7</b>	
	<b>3</b>	<b>0.0</b>	
	<b>4</b>	<b>0.8</b>	

## Intra-and Interday Precisions of AZY CS for Direct Assay :

<b>Std nominal conc.(mg/ml)</b>	<b>Validation day</b>	<b>Intraday mean (n=3) % RSD</b>	<b>Interday mean (n=4) % RSD</b>
<b>0.0025</b>	<b>1</b>	<b>2.4</b>	<b>4.1</b>
	<b>2</b>	<b>4.2</b>	
	<b>3</b>	<b>1.5</b>	
	<b>4</b>	<b>8.6</b>	
<b>0.005</b>	<b>1</b>	<b>8.5</b>	<b>4.1</b>
	<b>2</b>	<b>na</b>	
	<b>3</b>	<b>1.0</b>	
	<b>4</b>	<b>2.9</b>	
<b>0.25</b>	<b>1</b>	<b>1.4</b>	<b>1.5</b>
	<b>2</b>	<b>1.3</b>	
	<b>3</b>	<b>1.7</b>	
	<b>4</b>	<b>1.6</b>	
<b>1.0</b>	<b>1</b>	<b>1.1</b>	<b>2.0</b>
	<b>2</b>	<b>1.8</b>	
	<b>3</b>	<b>1.1</b>	
	<b>4</b>	<b>3.7</b>	

## Intra-and Interday Precisions of AZY QCs for Direct Assay :

<b>QC nominal conc.(mg/ml)</b>	<b>Validation day</b>	<b>Intraday mean (n=3) % RSD</b>	<b>Interday mean (n=4) % RSD</b>
<b>0.0111</b>	<b>1</b>	<b>7.8</b>	<b>7.3</b>
	<b>2</b>	<b>8.7</b>	
	<b>3</b>	<b>6.9</b>	
	<b>4</b>	<b>6.0</b>	
<b>0.111</b>	<b>1</b>	<b>0.5</b>	<b>1.4</b>
	<b>2</b>	<b>1.5</b>	
	<b>3</b>	<b>2.4</b>	
	<b>4</b>	<b>1.1</b>	
<b>1.11</b>	<b>1</b>	<b>0.8</b>	<b>1.4</b>
	<b>2</b>	<b>1.6</b>	
	<b>3</b>	<b>2.4</b>	
	<b>4</b>	<b>0.6</b>	

## Robustness Testing using Low QC:

Condition	Mean det'd conc. (mg/ml)	% RSD (for conc.)	Mean RT (min.)	% RSD (for RT)
30% ethanol, 3 ml/min	0.1060	0.5	11.09	0.9
32% ethanol, 3 ml/min	0.1054	1.6	9.40	0.3
28% ethanol, 3 ml/min	0.1027	2.7	12.02	0.4
30% ethanol, 3.15 ml/min	0.1119	0.9	10.48	0.3
30% ethanol, 2.85 ml/min	0.0992	1.0	11.91	0.1

## Summary of Results from AZY Direct Assay by SFC :

- Method selective for the direct aq. assay of meglumine formulation
- Validated over 4 days
- Linear response between 0.025-1 mg/mL with  $r = 0.9993$
- Sensitive (~1% chiral impurity could be detected)
- No sample pretreatment, no special mobile phase or additive
- Intraday ( $\pm 6\%$ ) and interday ( $\pm 3\%$ ) accuracies for CS
- Intraday ( $\pm 5\%$ ) and interday ( $\pm 2\%$ ) accuracies for QCs
- Intraday ( $\pm 9\%$ ) and interday ( $\pm 4\%$ ) precision for CS
- Intraday ( $\pm 9\%$ ) and interday ( $\pm 7\%$ ) precision for QCs
- DL and QL 1.5  $\mu\text{g/mL}$  and 5  $\mu\text{g/mL}$  respectively
- Robust method based on mp comp ( $\pm 2\%$ ) and flow rate ( $\pm 5\%$ )
- All SST criteria fulfilled in all 4 days of validation

## **Conclusions from Direct Aq. Formulation Assay by SFC :**

- Chiral SFC has been demonstrated to be applicable for neat aqueous formulation assays of pharmaceutical compounds, using AZM (basic) and AZY (acidic) as model compounds.
- This potentially could reduce the sample processing steps in R&D (e.g.- eliminate the necessity for dilution in organic solvent).
- The technique has potential wide applications based on success with both acidic and basic compounds.
- A generic method can be employed based on similarity of instrumental set up for both acidic and basic compounds.
- The method is equally successful for various commonly employed preformulation vehicles (lactic acid, meglumine) and at various pHs.
- The direct assay technique was validated to demonstrate its suitability for routine use.
- No adverse effect on chromatography or column life based on the 3 month study and >250 injections.
- Comparable QL and DLs with both compounds using the technique. <1% chiral impurity detected reliably. It is possible to further lower the QL by optimizing the chromatography.

## **Future Directions of the Direct Aq. Formulation Assay Technique :**

- Make other AZ relevant separation scientists aware of this technique
  - Already starting to gain popularity in other projects
- Employ an automated SFC method development using 6 column, 4 solvent approach for aq formulation assays of AZ compounds
- Demonstrate the applicability of direct aq formulation assay to 20 marketed formulations of racemic drugs
- Generate a knowledge based database on direct assay
- Template methods, collaboration with vendors (ChiralTech)

## References on Direct SFC assay of Aq Formulations :

- [1]. P. Mukherjee, S. Cook, Journal of Pharm. Biomed. Anal. 41 (2006) 1287-1292
- [2]. P. Mukherjee, Journal of Pharm. Biomed. Anal. (2007) 43 464-70
- [3]. O. Gyllenhaal, Journal of Chromatogr. A 1042 (2004) 173-180
- [4]. O. Gyllenhaal, Journal of Pharm. Biomed. Anal. 40 (2006) 971-974

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