

Evaluation of Evolute vs. Oasis Extraction Technologies for Bioanalytical Sample Preparation: Application to Acidic, Neutral, and Basic Compounds

Jacqueline Killmer

jacqueline.killmer@mpiresearch.com

ASMS Biotage Breakfast Meeting

June 2, 2009



Overview

- Introduction
- Extractions
 - Dyphylline
 - Fenofibric Acid
 - Midodrine and Desglymidodrine
 - Fluticasone Propionate
- Conclusions



Solid Phase Extraction

- Solid Phase Extraction (SPE)
 - Reversed Phase
 - Mixed Mode Ion Exchange
- Reduce matrix effects
- Reduce complexity of chromatography
- Concentrate sample

SPE Sample Prep

Hydrophobic

- Spike sample with internal Standard (IS) & dilute
- Load sample onto equilibrated column
- Wash away interferences
- Elute analyte



Mixed Mode Ion Exchange

- Spike sample with internal Standard (IS), dilute and ionize analyte
- Load sample onto equilibrated column
- Wash away neutral interferences
- Deionize & Elute analyte(s)

Evolute vs. Oasis

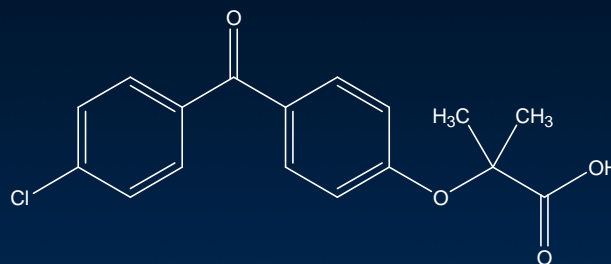
- Both are polystyrene divinylbenzene polymers
- ABN and HLB have a hydrophobic surface with polar character.
- CX and MCX are both functionalized with sulfonic acid

Types of Molecules

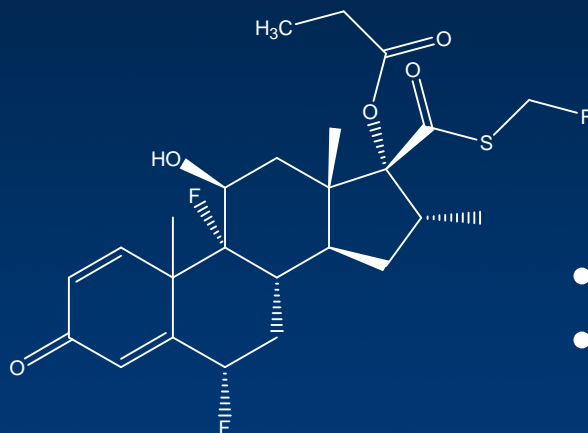
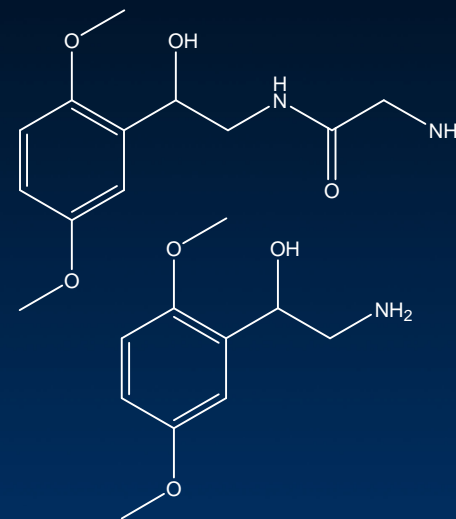
- Neutral
- Hydrophilic



- Acidic
- Lipophilic



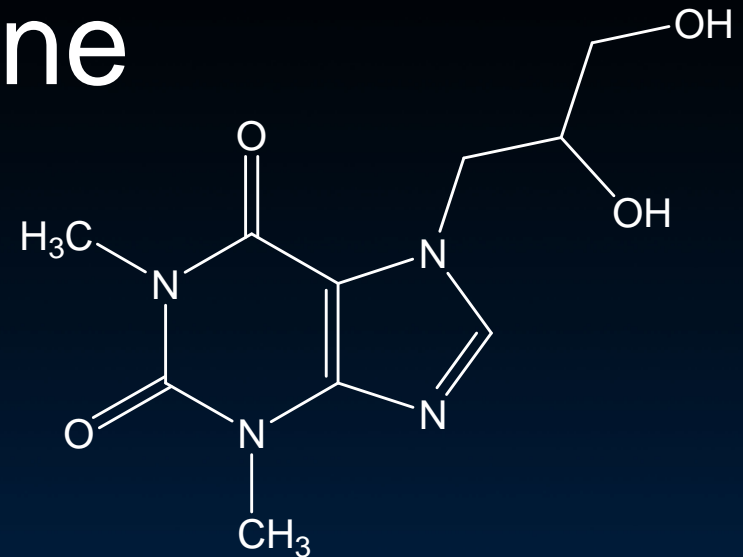
- Basic
- Hydrophilic



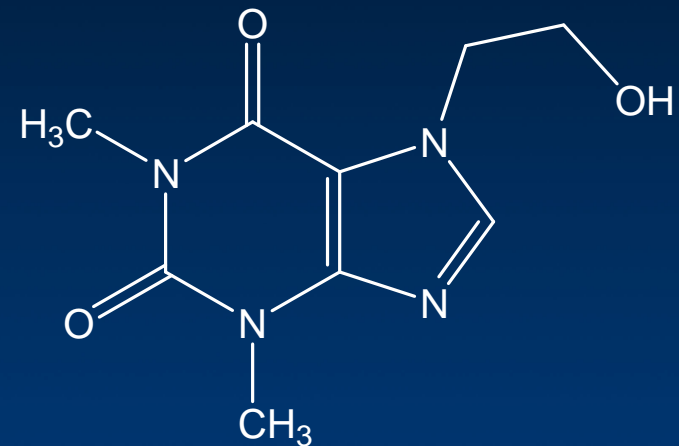
- Neutral
- Lipophilic

Dyphylline

- Broncodilator
- Originally approved in 1951
- Related to caffeine
- Neutral compound
- Hydrophilic compound
- $\text{Log } P = -1.9$
- Range = 0.2-200 ng/mL



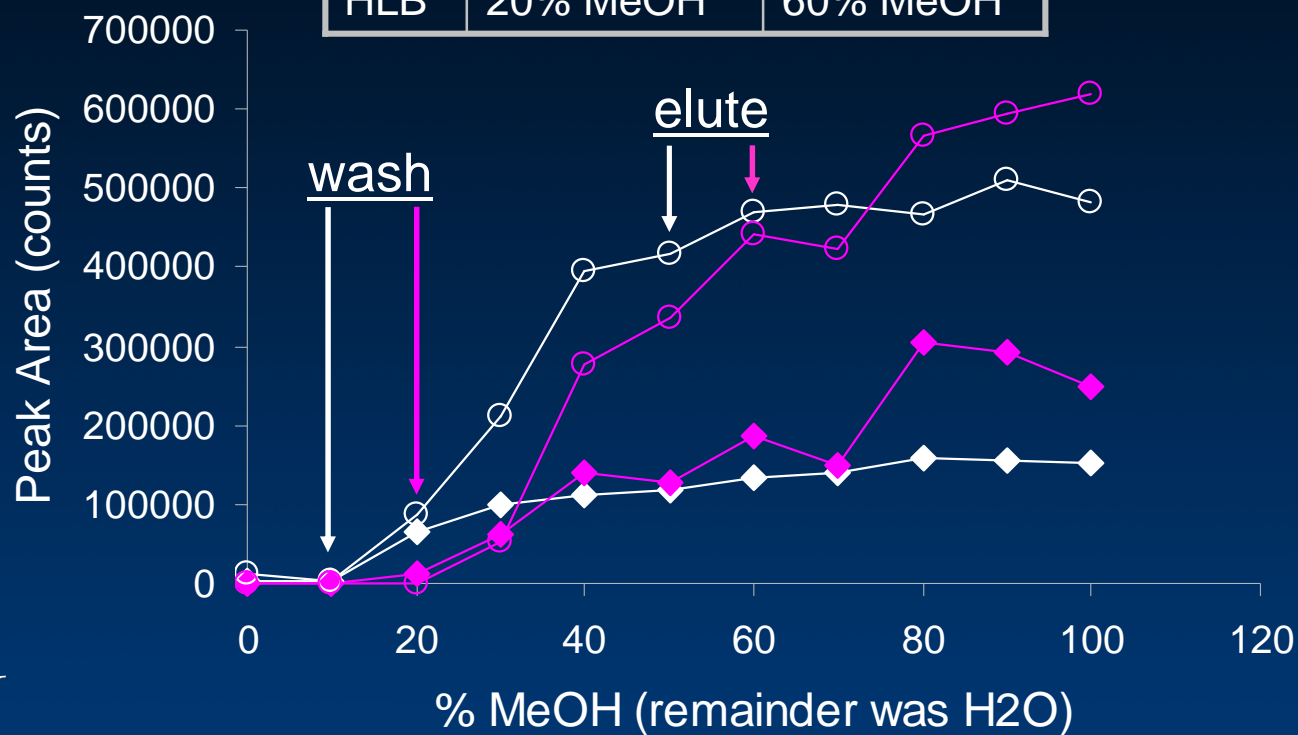
Dyphylline



7-(β-hydroxyethyl)theophylline

Dyphylline Extraction Optimization

	Wash	Elute
ABN	10% MeOH	50% MeOH
HLB	20% MeOH	60% MeOH



Recovery of Dyphylline

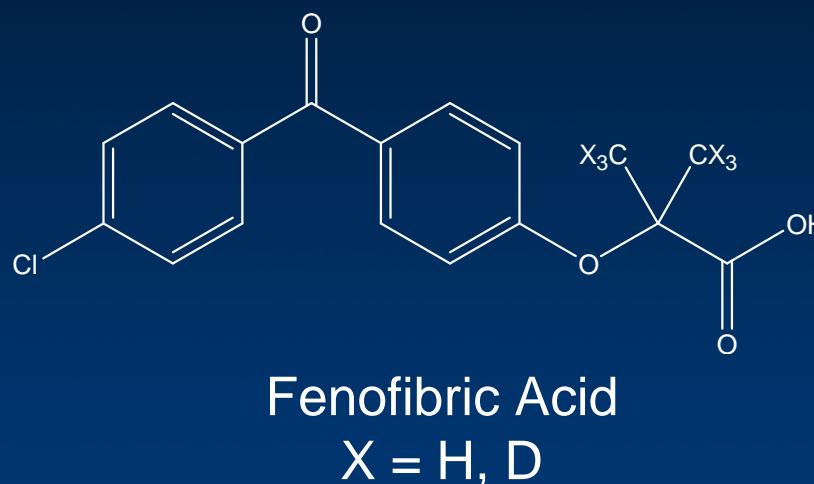
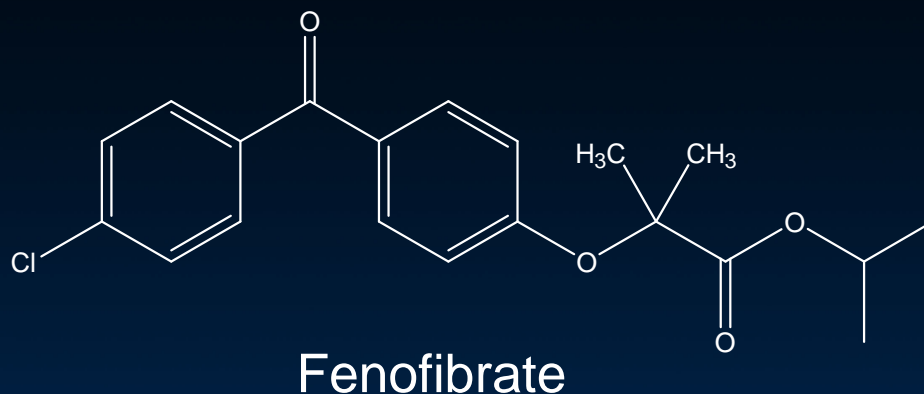
Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
46367	31663	38603	3882200	2465330	3447850	12929400	9041010	11428000
	32895	40518		2171400	3044700		8508830	10983100
	27638	37520		2280850	2728530		7005160	
Average	30732	38880		2305860	3073693		8185000	11205550
St Dev	2750	1518		148552	360535		1055851	314592
%RSD	9%	4%		6%	12%		13%	3%
%Recov	66%	84%		59%	79%		63%	87%

Dyphylline Calibration Curves

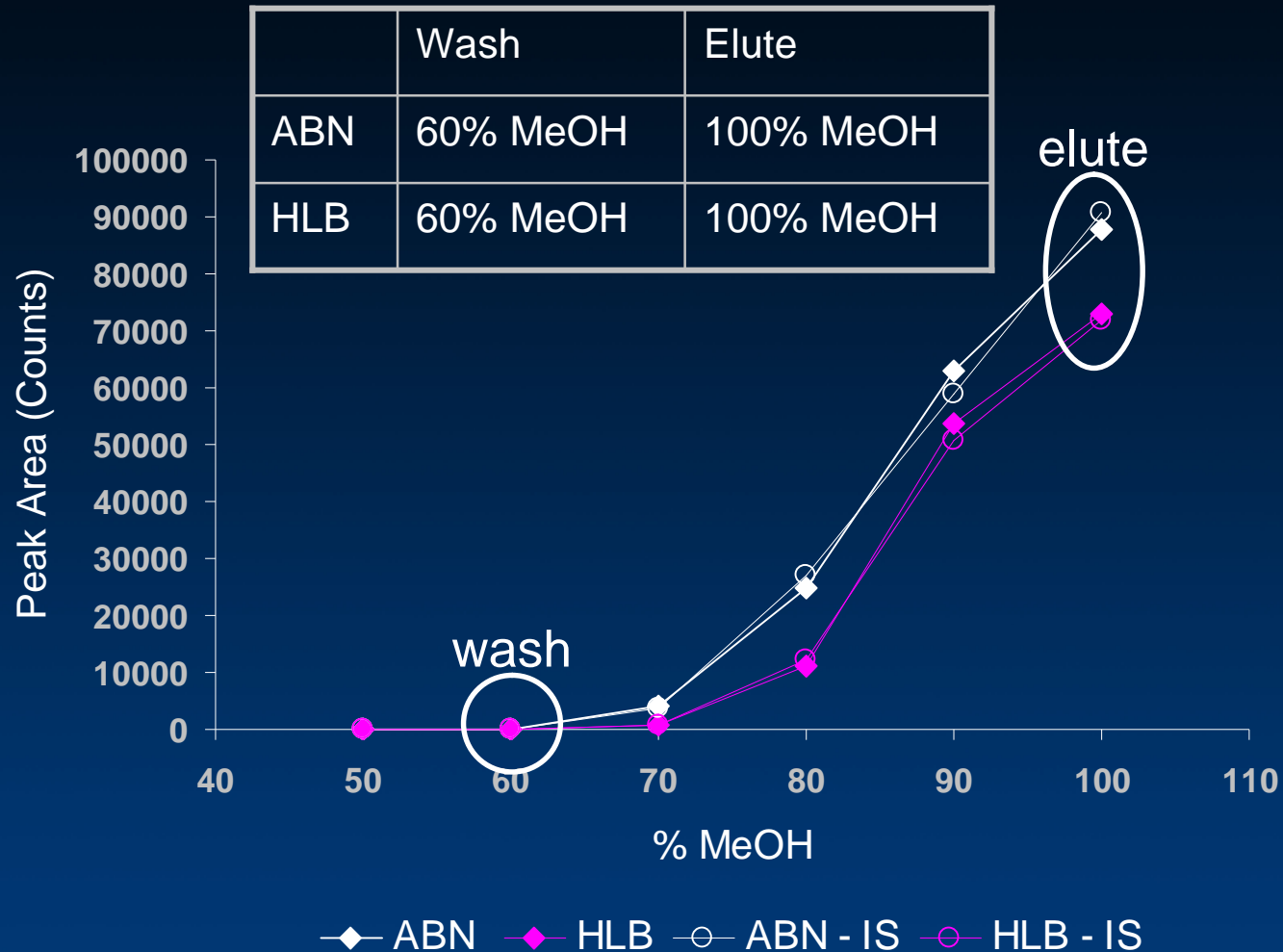
	Recovery	ABN	HLB
Intercept	0.00126	0.00674	0.00912
Slope	0.048	0.0538	0.0655
Correlation Coefficient	0.9967	0.9917	0.9955

Fenofibric Acid

- Antilipemic drug
- Approved in 1993 (Abbott)
- Hydrolyzed by esterases to fenofibric acid, the active metabolite
- Acidic compound
- Lipophilic compound
- Range = 10 -10,000 ng/mL



Fenofibric Acid Extraction Development



Recovery of Fenofibric Acid

	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	5817	4936	4334	85068	82216	68744	1405230	1213550	1214070
	5890	4527	4161	92141	82150	74097	1440930	1249820	1222740
	4831	4655	4246	91197	80374	78217	1475170	1243750	1203780
	4923	4863	4558	98575	86136	82449	1487200	1256840	1219100
	5013	4710	4352	90789	75670	77510	1381710	1326720	1380000
	5169	4764	4498	92272	81879	76427	1493920	1314970	1232830
Average	5274	4743	4358	91674	81404	76241	1447360	1267608	1245420
StDev	463	147	149	4309	3399	4580	46162	43957	66625
%RSD	9%	3%	3%	5%	4%	6%	3%	3%	5%
%Recov		90%	83%		89%	83%		88%	86%

Fenofibric Acid Calibration Curves

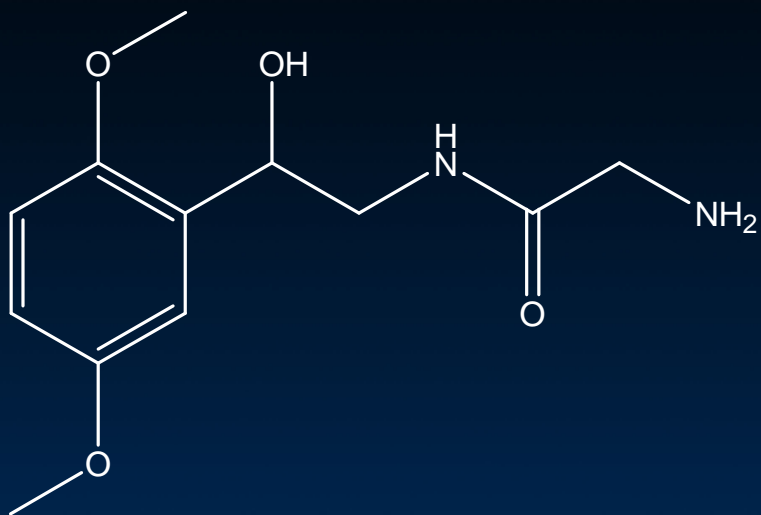
	Recovery	ABN	HLB
Intercept	0.0000417	0.00365	0.00252
Slope	0.00117	0.00121	0.00123
Correlation Coefficient	0.9998	0.9997	0.9995

Midodrine and Desglymidodrine

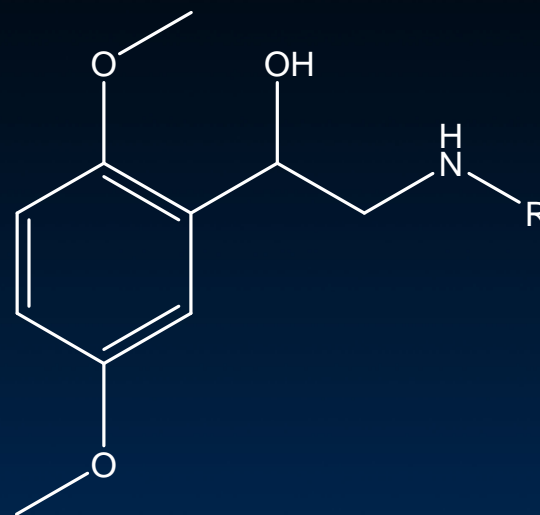
- Adrenergic alpha agonist used as a vasoconstrictor for the treatment of hypotension
- Approved in 1996 (Shire)
- Desglymidodrine is the active metabolite
- Basic compounds
- Lipophilic compounds
- Range = 0.1 – 50 ng/mL



Midodrine and Desglymidodrine



Midodrine
 $pK_a = 7.8$
 $\text{Log } P = -0.49$

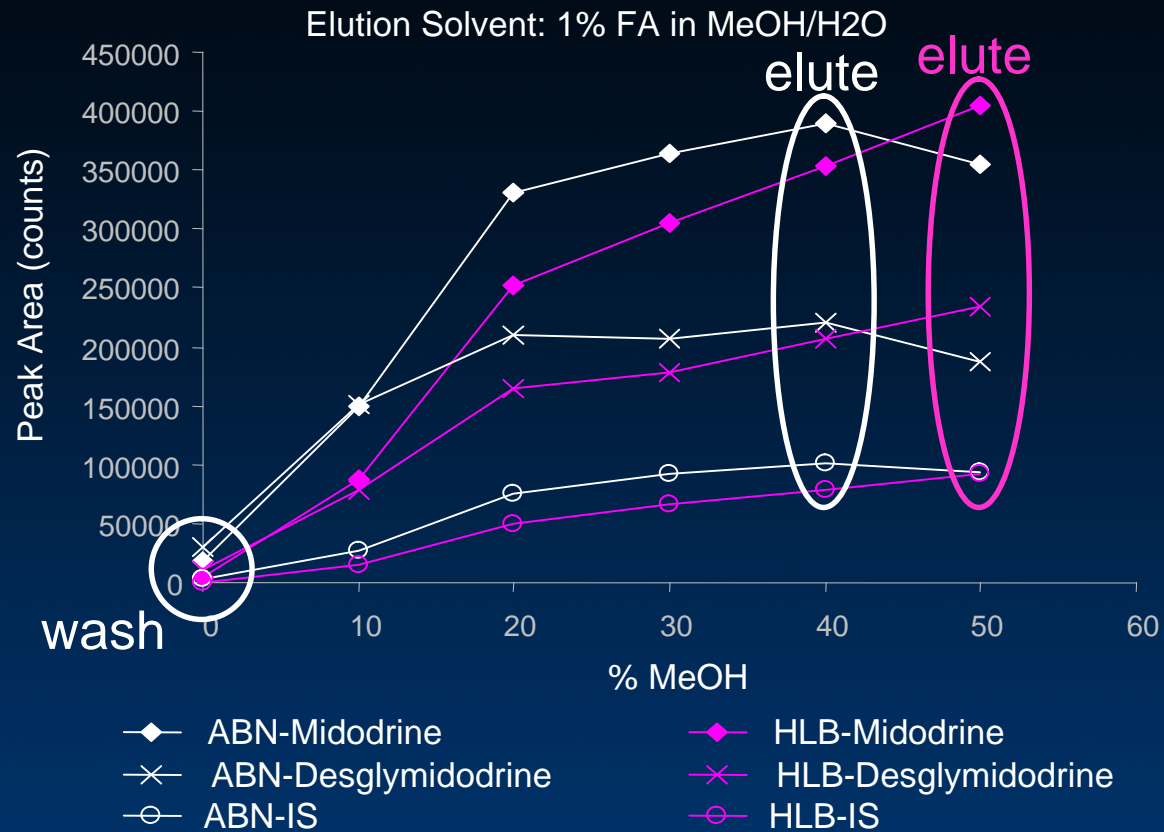


R = H, Desglymidodrine
CH₃, Methoxamine (IS)
 $pK_a = 9^*$
 $\text{Log } P = 0.3^*$

Midodrine and Desglymidodrine Method Development Strategy

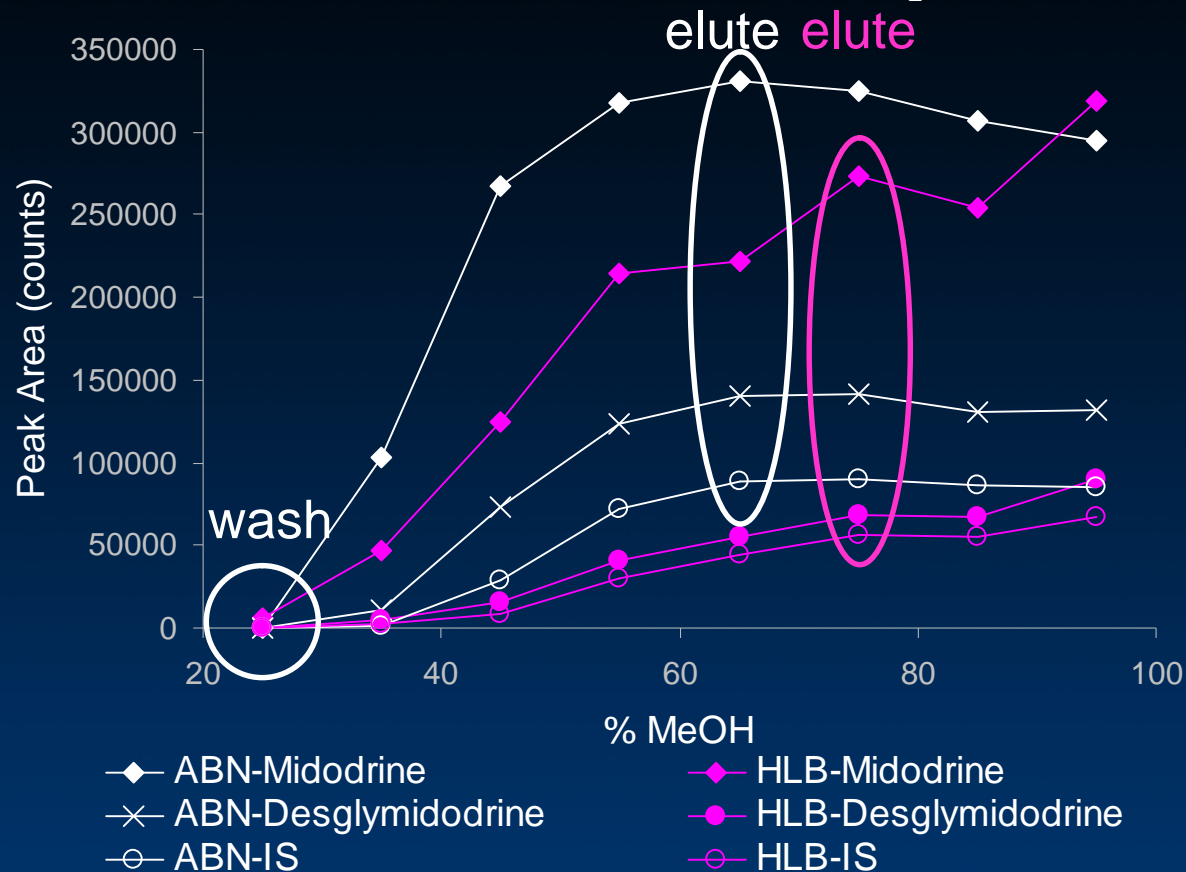
- Reversed Phase Mechanism
 - ABN or HLB
 - Acidic or Basic loading conditions
- Mixed Mode Cation Exchange
 - CX or MCX
 - Acidic loading
 - Basic elution

Midodrine & Desglymidodrine Extraction Development: ABN & HLB



Midodrine & Desglymidodrine Extraction Development: ABN & HLB

Elution Solvent: 5% NH₄OH in MeOH/H₂O




	Wash	Elute
ABN	0% MeOH	40% MeOH
HLB	0% MeOH	50% MeOH

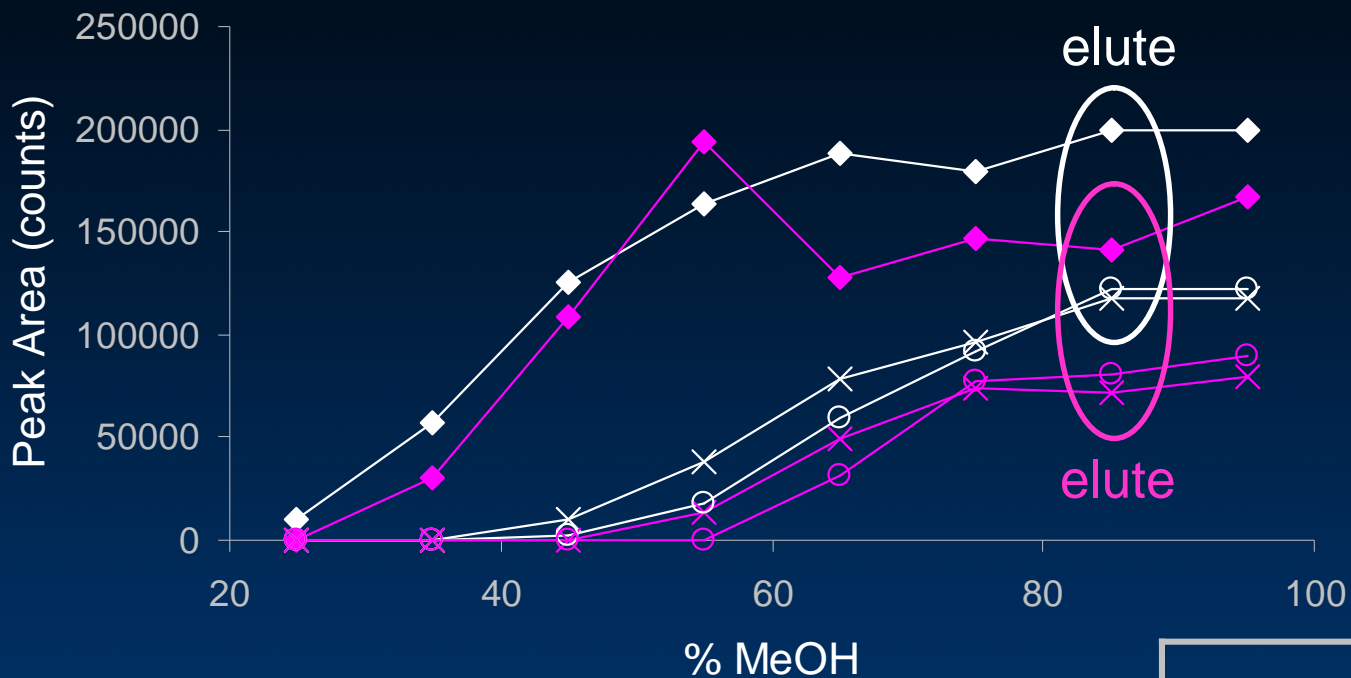
Recovery Midodrine: ABN vs. HLB

	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	38434	24935	20512	468130	292110	263358	4452750	2696240	2785650
	39694	27241	20182	451620	306515	258667	4359950	2898350	2699760
	37631	24336	23914	457222	307221	261858	4177570	3001380	2709210
	38496	25486	21962	457886	288338	271319	4308600	3044510	2745630
	38725	25155	21439	455349	292296	269597	4241580	2978630	2766450
	38778	24560	22290	469888	298757	255415	4161250	2936810	2749890
Average	38626	25285	21716	460016	297540	263369	4283617	2925987	2742765
StDev	666	1043	1349	7320	7968	6159	112289	123424	32968
%RSD	2%	4%	6%	2%	3%	2%	3%	4%	1%
%Recov		65%	56%		65%	57%		68%	64%

Recovery of Desglymidodrine: ABN vs. HLB

	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	18098	8608	4841	210873	103137	54045	2027390	947615	611329
	17846	8418	4127	209457	106330	55192	1975460	1011610	532768
	17930	8114	5429	209779	106540	53477	1883240	1020150	557949
	16937	8491	4732	211279	100729	55095	1951790	1067660	587778
	17509	8191	4452	206673	103839	56083	1941400	1030290	573669
	17753	7928	4585	213209	103993	54623	1943840	1018230	549906
Average	17679	8292	4694	210212	104095	54753	1953853	1015926	568900
StDev	413	257	437	2184	2159	919	47160	38964	28162
%RSD	2%	3%	9%	1%	2%	2%	2%	4%	5%
		47%	27%		50%	26%		52%	29%

Midodrine and Desglymidodrine Extraction Development: CX & MCX



	Elute
CX	85% MeOH
MCX	85% MeOH

Recovery of Midodrine: CX vs. MCX

	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	36214.2	24117	2435	433425	333921	314991	4211590	3221700	3140880
	36138.1	24806	23609	430189	340139	315369	4212090	3322160	3169480
	35694	25462	22980	431222	339169	318065	4125050	3301970	3133540
	35231.5	24995	22800	433650	338317	315542	4144840	3317780	3189940
	36286.2	24892	23326	438247	343636	320873	4242380	3323350	3173650
	36287.1	26493	23080	454349	344442	299182	4183620	3429740	3068710
Average	35975	25127	23358	436847	339937	314004	4186595	3319450	3146033
StDev	427	797	563	9013	3832	7597	44557	66408	43363
%RSD	1%	3%	2%	2%	1%	2%	1%	2%	1%
%Recov		70%	65%		78%	72%		79%	75%

Recovery of Desglymidodrine: CX vs. MCX

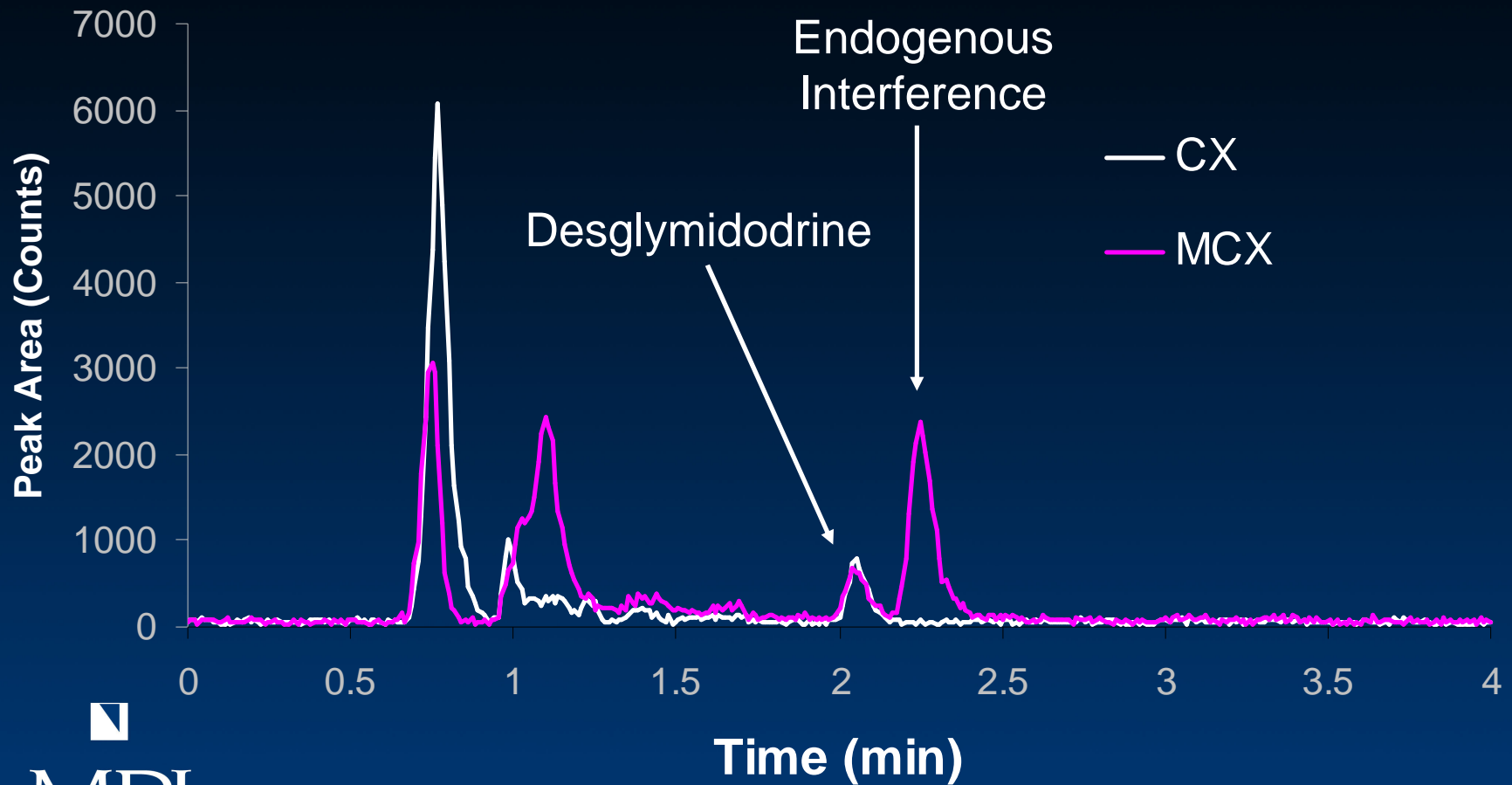
	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	15834.4	9142.85	8301.99	198294	120263	115268	1930020	1260340	1149040
	16661.8	9569.02	8495.61	191966	126112	114965	1904520	1208660	1160020
	16501.1	9953.09	8295.83	199798	131651	113850	1896580	1193080	1151050
	15728.8	9323.59	8327.64	198429	129541	112114	1929930	1213740	1169390
	16160.1	9125.1	8048	202978	123296	114003	1970280	1206070	1169740
	17944.1	9905.92	8482.09	210943	130966	105888	1948580	1224350	1156600
Average	16472	9503	8325	200401	126972	112681	1929985	1217707	1159307
StDev	807	367	162	6287	4554	3507	27327	23243	8854
%RSD	5%	4%	2%	3%	4%	3%	1%	2%	1%
%Recov		58%	51%		63%	56%		63%	60%

Midodrine and Desglymidodrine Calibration Curves

	Neat	ABN	HLB	Neat	CX	MCX
Intercept	0.000213	0.00147	0.00352	0.00225	0.00149	0.000397
Slope	0.0956	0.0987	0.134	0.0944	0.104	0.105
Correlation Coefficient	0.9976	0.9946	0.996	0.9995	0.9998	0.9992

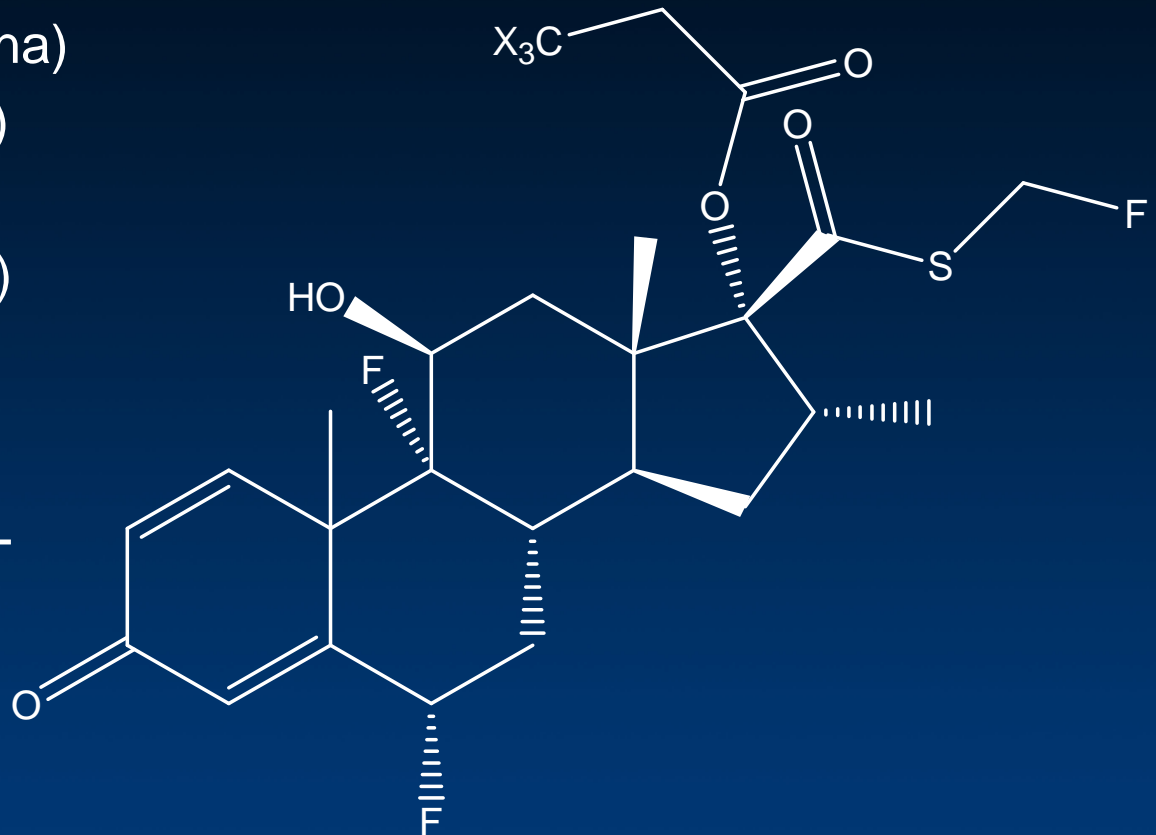
	Neat	ABN	HLB	Neat	CX	MCX
Intercept	0.00027	0.00224	0.000549	0.00108	0.000498	0.0000473
Slope	0.0479	0.0356	0.0279	0.0458	0.0383	0.0373
Correlation Coefficient	0.9988	0.9904	0.9993	0.9996	0.9993	0.9998

Evolute CX vs. Oasis MCX Chromatograms



Fluticasone Propionate

- Synthetic corticosteroid for the treatment of dermatitis, psoriasis, rhinitis, and asthma
- Approved in:
 - Cutivate – 1990 (Altana)
 - Flovent – 1996 (GSK)
 - Advair – 2000 (GSK)
 - Flonase - 1994 (GSK)
- Neutral compound
- Lipophilic compound
- Range = 3 - 300 pg/mL




Fluticasone Method Development Strategy

- Reversed Phase Mechanism
 - ABN or HLB
 - Acidic loading conditions
 - Basic loading conditions
 - Reduce endogenous acidic compounds
 - Cleaner chromatography

Fluticasone Extraction Development



Recovery of Fluticasone

	Low Recovery	ABN QC Low	HLB QC Low	Mid Recovery	ABN QC Mid	HLB QC Mid	High Recovery	ABN QC High	HLB QC High
	1,542	4,527	10,396	10,830	32,877	14,169	45,690	108,193	43,625
	1,936	4,365	6,902	11,962	33,482	11,897	49,739	117,360	44,152
	2,426	3,660	6,812	11,709	29,431	12,849	54,402	89,055	41,563
	3,278	6,272	2,968	17,808	17,375	9,655	127,911	59,620	40,387
	4,890	5,682	2,432	21,370	18,000	9,754	136,905	56,870	42,110
	4,536	5,835	4,585	27,968	17,443	10,426	150,799	52,229	40,165
Average	3,101	5,057	5,682	16,941	24,768	11,458	94,241	80,554	42,001
StDev	1,381	1,018	2,970	6,804	7,969	1,827	49,148	28,257	1,640
%RSD	45%	20%	52%	40%	32%	16%	52%	35%	4%
		163%	183%		146%	68%		85%	45%

Fluticasone

Comparison of Calibration Curves

	Recovery	ABN	HLB
Intercept	-0.0103	0.00526	0.0135
Slope	0.0296	0.0122	0.0118
Correlation Coefficient	0.999	0.997	0.996

Conclusions

- Optimization of SPE methods based on an understanding of the properties of the analyte improve recoveries and reduce endogenous interferences.
- The Evolute ABN phase is slightly more polar than the Oasis HLB phase.
- Recoveries are typically higher with the Evolute ABN and CX SPE phases.
- Precision of the Evolute phases is similar to that of the Oasis phases.



Acknowledgements

Abram Brubaker
David Bailey
Jonathan Bissland
Jennifer Brown
Joshua Ehrsam
Stephanie Graham
Renaë Huyser
Justin Koehler
Melinda Maile
Erik Rodgers
Ly Tran
Julia Zamler

Bernard Ntsikoussalabongui
Dhruba Bhattarai
Holly Bridges
Christopher Ciptadjaya
Troy Frayer
David Humphries
Safaa Kakos
Tina Martin
Beauregard Mason
Bob Texas
Katherine Wozniak

For conditions for any of the assays in this presentation, please contact me at jacqueline.killmer@mpiresearch.com

