A New Polymer-based SPE Sorbent to Reduce Matrix Effects in Bioanalytical LC-MS/MS

M. Cleeve, L. Williams, A. Howells, G. Davies, S. Merriman, S. Plant, O. Gooding,
J. Labadie, R. Johnston, M. Burke[1], R. Calverley, H. Lodder, C. Desbrow

Argonaut Technologies Ltd. now a Biotage company, New Road, Hengoed, Mid Glamorgan, CF82 8AU, UK
marketing_info@biotage.com
[1] MFB Consulting LLC, 5560 N. Via Velazquez, Tucson, AZ 85715

Introduction

Sample preparation is essential when analyzing drugs in biological fluid samples (e.g. plasma and urine), even when selective analytical techniques such as LC-MS/MS are used. Increasingly, bioanalytical scientists utilize solid phase extraction (SPE) as the preferred sample preparation technique for the extraction of drugs and their metabolites from biological fluids. Recent years have seen a resurgence in the use of resins as SPE sorbents. Resins have been advocated for extractions of this type because compared with silica based SPE sorbents they generally show improved recovery of polar analytes and the ability to simultaneously extract large numbers of analytes with different functionalities simultaneously.

However, the current generation of resin based SPE products, used with generic methodology, can produce dirty extracts which lead to matrix effects such as ion suppression or enhancement in LC-MS/MS analysis. These are due to high levels of co-extracted endogenous material that are transferred from the sample to the final extract during the extraction process. At Argonaut Technologies we have focussed on the development of a resin based SPE product that can deliver the benefits of resin based SPE (for example extraction of a broad range of analytes using a single generic method) without the compromise of dirty extracts.

This poster describes the use of EVOLUTE™ ABN (Acidic Basic Neutral) Sample Preparation Products for extraction of drugs from biological fluids. The main topics are:

1. Extraction of a broad range of analytes using a single generic method

2. Improved extract cleanliness resulting in reduced ion suppression

EVOLUTE Sample Preparation Products

1. Extraction of a Broad Range of Analytes Using a Single Generic Method

EVOLUTE ABN is a polystyrene-divinylbenzene based material, surface modified with hydroxyl-functional oligomers, which impart water-wettable characteristics. This allows simultaneous extraction of acidic, basic and neutral compounds with a wide polarity range from aqueous biological fluid samples using a single generic method. This is illustrated using a probe set selected for its diversity of structure, functionality and polarity.

Argonaut Biotage

EXPERIMENTAL CONDITIONS

Sample: Human plasma spiked with probe analytes, as listed in **Table 1**.

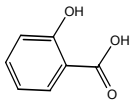
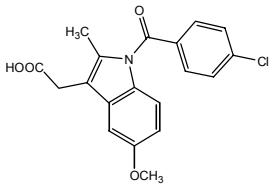
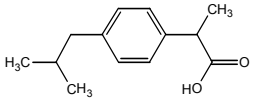
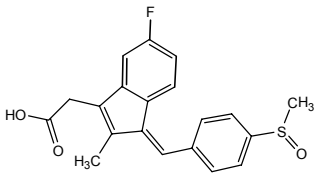
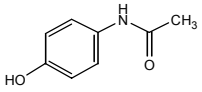
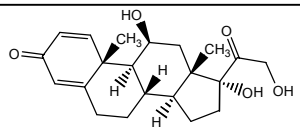
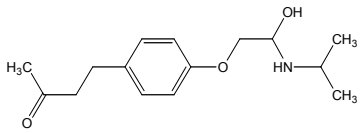
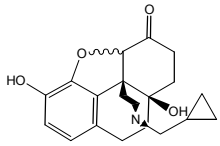
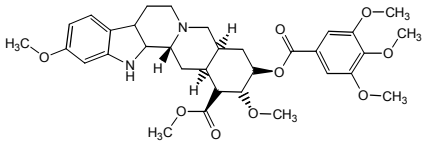
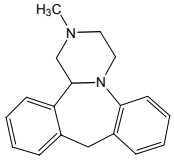
Classification	Compound	Structure	logP[1]	pK[1]	Plasma Sample Concentration (pg/µL)
Acidic, polar	Salicylic Acid[2]		2.24	2.9	50 pg
Acidic, medium polarity	Indomethacin		3.10	4.3	5 pg
Acidic, medium polarity	Ibuprofen		3.51	5.2	50 pg
Acidic, non-polar	Sulindac		3.59	3.6	5 pg
Neutral, very polar	Acetaminophen		0.34	N/A	50 pg
Neutral, polar	Prednisolone		1.49	N/A	50 pg
Basic, polar	Metoprolol		1.34	10.8	5 pg
Basic, polar	Naltrexone		1.80	9.2	5 pg
Basic, non-polar	Reserpine		3.11	6.6	50 pg
Basic, non-polar	Mianserin		3.67	8.3	50 pg

Table 1. Probe compounds used for recovery experiment. Note: Salicylic acid extracted from buffer due to its presence in blank plasma samples.

[1] pK and logP values were obtained from literature or values were calculated if not available.

100 µL plasma sample extracted using EVOLUTE ABN 25 mg/1 mL columns and SPE method as described in Experimental Conditions.

EXPERIMENTAL CONDITIONS

EVOLUTE ABN Generic Method

SPE Column: EVOLUTE ABN 25 mg/1 mL (p/n 600-0002-A)
Sample Pre-treatment: Dilute plasma sample 1:3 (v/v) with aqueous formic acid (1 %, v/v). Mix thoroughly.
Conditioning: Condition each column with methanol (1 mL).
Equilibration: Equilibrate each column with aqueous formic acid (0.1%, v/v, 1 mL).
Sample Load: Load sample (100 µL diluted plasma).
Interference Wash: Elute interferences with water/methanol (95:5, v/v, 1 mL).
Analyte Elution: Elute analytes with methanol (500 µL).
Post-extraction: Extracts were evaporated to dryness and reconstituted in mobile phase.

HPLC
Guard Column: Eclipse XDB-C18 (2.1 x 12.5 mm, 5 µm)
Analytical Column: Eclipse XDB-C18 (2.1 x 50 mm, 3.5 µm)
Mobile Phase: A = 0.1% (v/v) formic acid, B = acetonitrile
Gradient:

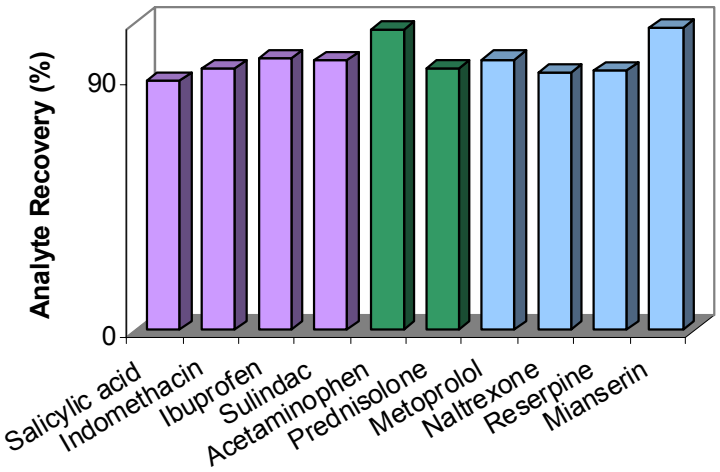
Time	% B (Acetonitrile)
0	20
5	36
7	70
8	70
8.1	20

Flow Rate: 0.25 mL/minute
Injection Volume: 50 µL
Temperature: 30 °C

MS/MS
Instrument: Varian 1200L triple quadrupole
Ionization: Electrospray, +ve (-ve for salicylic acid)
Drying Gas Temperature: 300 °C

Analyte	MRM Transition	Collision Energy (eV)
Salicylic acid	137.1>93.2	15.5
Indomethacin	358.1>139.0	-19.5
Ibuprofen	207.1>161.2	-9.5
Sulindac	357.2>233.0	-43.5
Acetaminophen	152.1>110.0	-15.0
Prednisolone	361.3>147.0	-20.0
Metoprolol	268.1>116.0	-16.5
Naltrexone	342.2>324.0	-18.5
Reserpine	609.5>195.0	-33.5
Mianserin	265.2>208.0	-19.0

Analyte	Recovery (%, n=5)	RSD (%)
Salicylic acid	90	3.6
Indomethacin	94	5.3
Ibuprofen	98	3.7
Sulindac	97	5.8
Acetaminophen	108	5.1
Prednisolone	94	0.75
Metoprolol	97	3.5
Naltrexone	92	4.3
Reserpine	93	7.9
Mianserin	109	5.7



EVOLUTE ABN delivers high absolute recoveries (>90 %) with excellent reproducibility (0.75-7.9 % rsd, n=5) for a wide range of compounds

Comparison with other Commercially Available Resin Based SPE Products

The applicability of EVOLUTE ABN for extraction of a range of acidic, basic and neutral compounds from plasma was compared with other commercially available resin based SPE products.

EXPERIMENTAL CONDITIONS

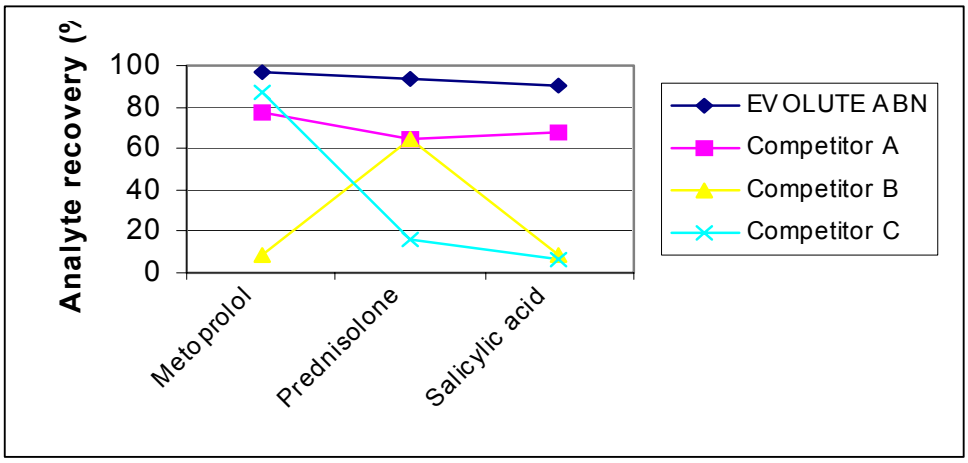
SPE Method

EVOLUTE ABN: EVOLUTE ABN generic method, as described on page 3.
Competitors A, B, C: Manufacturers recommended generic methods were used.

Analytical Method

As described above.

RESULTS



CONCLUSIONS

- Using a single generic SPE method, EVOLUTE ABN can be used to extract a wide range of acidic, basic and neutral compounds from plasma samples.
- High recoveries (>90%) with low variation (rsd <8%) were achieved when extracting probe compounds spiked in the concentration range 5-50 pg/µL.
- EVOLUTE ABN showed consistently higher recoveries than competitor resin based SPE products for a representative probe set.

---

2. Comparison of Extract Cleanliness

EVOLUTE ABN has an optimized pore structure designed to minimize the retention of endogenous plasma components during the solid phase extraction process, and produce cleaner extracts for analysis.

HPLC-UV was used to investigate the relative cleanliness of blank plasma extracts produced using EVOLUTE ABN, and a competitor resin based SPE product (A and C).

The majority of bioanalytical samples are analyzed by LC-MS/MS. So in addition, the effect of increased extract cleanliness on reduction of ion suppression caused by plasma extracts was investigated using LC-MS/MS flow injection analysis (as described by Bonfiglio et al [1]).

EXPERIMENTAL CONDITIONS

Sample Preparation

Blank human plasma samples (500 µL) were extracted using the EVOLUTE ABN generic method, as described on page 3. As a comparison, plasma samples from the same pooled master batch of plasma were extracted using competitor resin based SPE products, using the manufacturer's recommended generic method.

a. Comparison of Extract Cleanliness using HPLC-UV Analysis

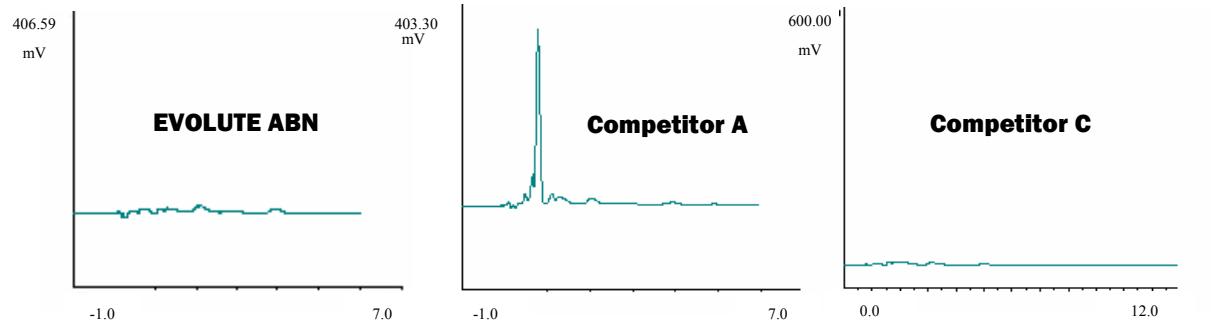
Following extraction, extracts were evaporated to dryness and reconstituted in mobile phase (500 µL).

HPLC Conditions

Column: Genesis® C18, 15 cm x 4.6 mm, 4 µm
Mobile Phase: 60:40 (v/v) phosphoric acid (0.2%, pH 2.5) : methanol containing 200 µL diethylamine
Flow Rate: 1.4 mL/minute
Injection Volume: 40 µL
Wavelength: 220 nm
Temperature: 40 °C

RESULTS

The chromatograms generated are shown below. A range of extract cleanliness is observed with UV detection. Extracts produced using EVOLUTE ABN and Competitor C exhibit low levels of co-extracted material, whereas the extract produced using Competitor A contains significant levels of co-extracted material.



b. Effect of Extract Cleanliness on Ion Suppression

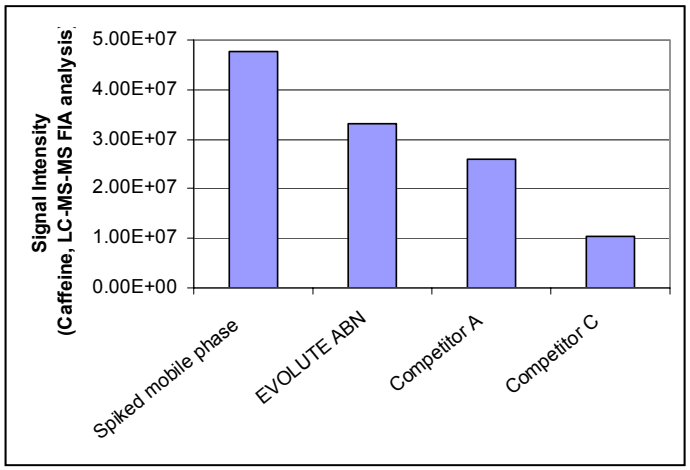
Following extraction, the plasma extracts were evaporated to dryness and reconstituted in mobile phase spiked with caffeine at a concentration of 1 µg/mL.
The MS/MS signal intensity observed from the spiked plasma sample extracts was then compared with that observed for pure mobile phase spiked at the same concentration (1 µg/mL) with caffeine.

FIA LC-MS/MS Conditions

Mobile Phase: Water:acetonitrile:methanol: 0.1% formic acid (50:45:5:1, v/v)
Flow Rate: 0.25 mL/minute
Injection Volume: 5 µL
Instrument: Varian 1200L triple quadrupole
Ionization: Electrospray, +ve
Drying Gas Temperature: 260 °C
SRM Transition for Caffeine: m/z 195>138

RESULTS

The signal intensity from spiked mobile phase relative to that from extracts produced using EVOLUTE ABN, competitor A and competitor C is shown below.



Sample	% Ion Suppression
Spiked mobile phase	0
EVOLUTE ABN	28
Competitor A	46
Competitor C	78

Compared to spiked mobile phase, extracts generated using EVOLUTE ABN suppress the signal from caffeine by only 28 %. Competitors A and C exhibit 46 % and 78 % ion suppression, respectively.

COMMENTS

EVOLUTE ABN produces cleaner extracts than competitor polymer-based SPE products. Extracts contain low levels of co-extracted material observed with HPLC-UV analysis, and a significant reduction in ion suppression effects, leading to an increased signal-to-noise ratio in LC-MS/MS.

For the competitor products, we have also shown that there is no direct correlation between extract cleanliness observed using HPLC-UV analysis, and matrix effects in LC-MS/MS analysis. For example, competitor C shows minimal levels of co-extracted material in HPLC-UV, while the LC-MS/MS signal exhibits 78 % ion suppression. This suggests that different resin based SPE products remove different matrix components from the original plasma samples.

OVERALL CONCLUSIONS

EVOLUTE ABN can be used to extract a wide range of analytes from biological fluid samples with high recoveries and low rsd.

Biological fluid extracts produced using EVOLUTE ABN are cleaner, and give less ion suppression than those produced using competitor polymer-based SPE products.

References

1. R. Bonfiglio, R.C. King, T.V. Olah, K. Mwerkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175-1185.

Cardiff, Wales
New Road, Hengoed
Mid. Glamorgan.
CF82 8AU, UK
T: +44 (0) 1443 811811
F: +44 (0) 1443 816552
marketing_info@biotage.com