Evaluation of basic analytes using a novel hybrid reversed phase method for solid phase extraction by HPLC-MS

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The Microlute™ CP range from Porvair Sciences, offers a new method of solid phase extraction (SPE) using a unique hybrid polymer structure combined with retentive media. This composite design enhances the flow-through of samples throughout its porous structure to maximise interactions between analytes and the solid phase to deliver a highly reproducible SPE method. This application note demonstrates the robust SPE LC-MS methodology of the Microlute™ CP Reversed Phase (RP) SPE 30 mg plates on basic analytes by comparing recoveries and reproducibility benefits over loose-filled products.

Introduction

Solid phase extraction (SPE) is a sample preparation method for the clean-up of samples before analysis with HPLC or GC analysis. SPE offers a number of advantages to the analyst, including less system downtime and troubleshooting, cleaner chromatograms with a reduction of contaminating compounds, and more reproducible analyte recoveries. Traditionally, SPE methods use loose-filled resins which can create problems, such as voids in the sorbent beds leading to channelling, inconsistent flow-through of solutions, instability at extreme pHs and residual silanol activity. This materialises in less interactions between analytes with the active resin leading to inconsistent results and poor analyte recovery.

Over more recent years, significant advantages have been seen from using polymer-based materials. The processes used to synthesise polymer-based sorbents enable incorporation of numerous chemical functionalities into the porous framework (*Figure 1*). The ability to generate highly specific and regular functionality gives high retention capacity for different types of compounds including basic compounds and stability at extreme pHs.[1]

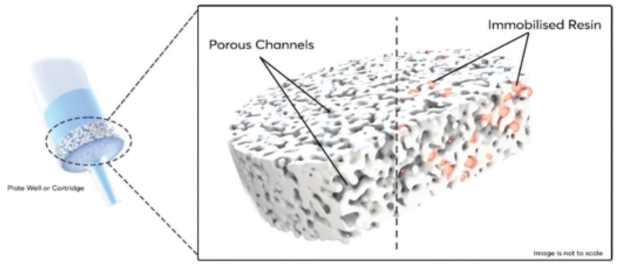

Figure 1. Microlute™ Hybrid Technology. A network of porous channels containing immobilised resins for solid phase extraction.

With only 20% of pharmaceutical analytes exhibiting acidic properties, basic analytes accounts for 70% of pharmaceutical compounds making them the most abundant analyte type being analysed via HPLC/GC. Unlike neutral and acidic compounds, basic compounds require less method development to ensure they are sufficiently retained on the sorbent medium.[2]

Retention of basic analytes from polar solutions onto reversed phase SPE materials occurs due to Van der Waal forces or dispersion forces between carbon-hydrogen bonds of the analyte and the functional groups bonded to the sorbent material. Stronger Van der Waal forces lead to greater retention on the reversed phase. Finally, silica-based resins can be sensitive to stationary phase collapse if the sorbent bed becomes dry after the condition step whereas, polymer-based sorbents are less susceptible to drying out. This enables quicker SPE method development and gives the analyst ease of mind when performing SPE. In addition, minor packing differences of sorbents into cartridges and wells can cause significant differences in the flow of solutions when performing SPE steps.

The outlined advantages of polymer-based sorbents greatly suggest a superior method of solid phase extraction not only in performance but increased reproducibility of recovery and retention of analytes of interest. To demonstrate the sensitivity and robustness of the 30 mg Microlute™ CP RP, an SPE experiment was performed using spiked aqueous sample matrices containing basic analytes and was compared against five commercially available 30 mg loose-filled reversed phase products.

Evaluation of Basic Analyte Recovery and Reproducibility

To highlight the sensitivity of the 30 mg RP composite, SPE was carried out on 12 wells using spiked aqueous sample matrices with eight basic analytes. 10 µg of each analyte was loaded onto each well, eluted with organic solvent, dried, and reconstituted before being diluted ready for analysis with LC-MS. The same technique was used to compare performance of equivalent competitor 30 mg plates.

Experimental

Chemicals:

Caffeine, atenolol, salbutamol, propranolol, nortriptyline, protriptyline, imipramine, desipramine, amitriptyline, formic acid, methanol, water, 35% ammonia solution.

Sample Preparation:

A stock of 1,000 µg/ml for each of the basic analytes was made in methanol. A basic load solution was made by using 500 µl stock solution and diluting to 50 ml with water containing 0.1% (v/v) ammonia solution.

Solid Phase Extraction Method:

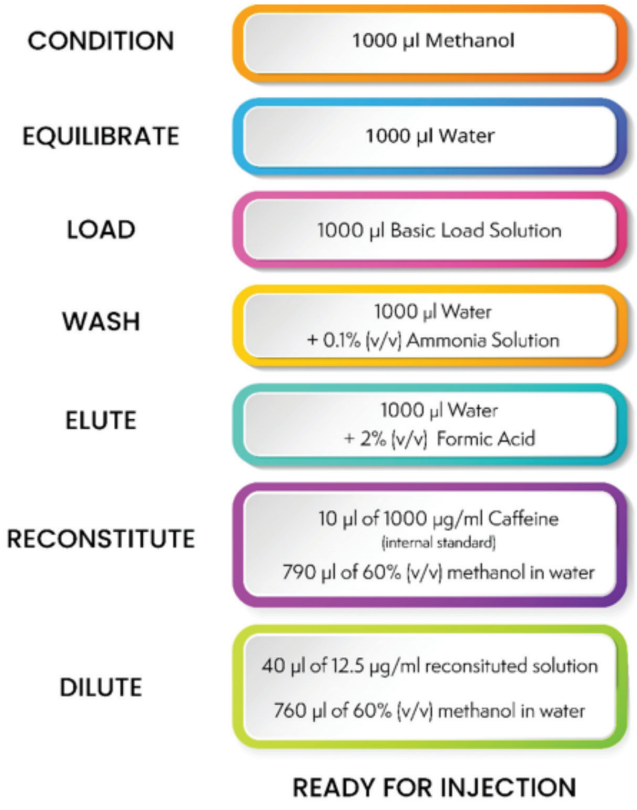

Figure 2. Flow schematic of solid phase extraction method for basic analytes.

Table 1. LC system conditions for chromatographic separation of basic analytes.

LC Conditions:

LC system	Agilent LC-MS, consisting of a 1260 LC and Single Quadrupole Mass Spectrometer.
Column	Raptor Biphenyl 30 x 2.1 mm, 1.8 µm
Column temp.	45°C
Injection volume	2.00 µL
Flow rate	600 µL/min
Mobile phase A	0.1% Formic acid in water
Mobile phase B	0.1% Formic acid in methanol
Solvent Composition	**Time (min) / A% / B%** 0.10 / 95.0 / 5.0 4.30 / 57.5 / 42.5 6.50 / 57.5 / 42.5 6.51 / 20.0 / 80.0 8.20 / 20.0 / 80.0 8.21 / 95.0 / 5.0 14.00 / 95.0 / 5.0

Table 2. Mass spectrometer conditions.

Parameter	Value
Gas Temperature	350 °C
Gas Flow	13 L/min
Nebuliser	30 psi
Capillary Voltage	4000 V
Fragmentor Voltage	100 V
Scan Type	SIM
Ion Mode	ESI

The dwell time (ms) varied depending on compound detection as followed:

Table 3. Dwell time values for basic analytes.

Compound	Dwell Time (ms)
Atenolol	70
Salbutamol	70
Caffeine	150
Propranolol	130
Imipramine	50
Amitriptyline	50
Desipramine	50
Protriptyline	50
Nortriptyline	50

Results and Discussion

Chromatogram

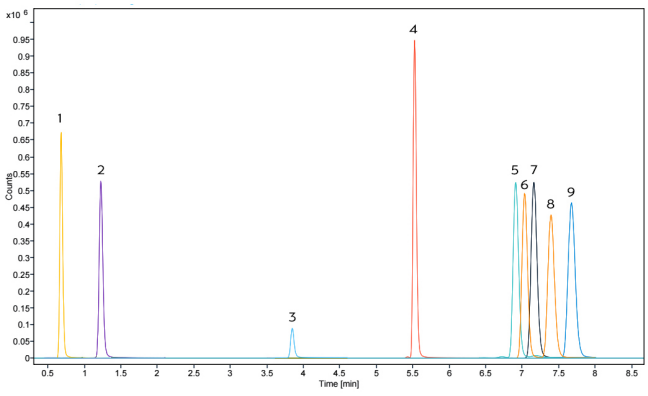

Figure 3. Chromatogram of basic analytes calibration standard. Peak assignments can be found in Table 4.

Peak Assignment

Table 4. Properties and retention times for the basic compounds analysed - a Predicted value from Pubchem [3].

Number	Compound	Type	R.T (min)	Formula	Molecular Mass	LogP[a]	pKa[a]
1	Salbutamol	Basic	0.65	$C_{13}H_{21}NO_3$	239.31	0.3	10.3
2	Atenolol	Basic	1.27	$C_{14}H_{22}N_2O_3$	266.34	0.2	10.4
3	Caffeine	ISTD	3.89	$C_8H_{10}N_4O_2$	194.19	-0.1	14.0
4	Propranolol	Basic	5.55	$C_{16}H_{21}NO_2$	259.34	3.0	9.4
5	Desipramine	Basic	6.80	$C_{18}H_{22}N_2$	266.4	4.9	9.6
6	Protriptyline	Basic	7.00	$C_{19}H_{21}N$	263.4	4.4	9.7
7	Imipramine	Basic	7.16	$C_{19}H_{24}N_2$	280.4	4.8	9.4
8	Nortriptyline	Basic	7.41	$C_{19}H_{21}N$	263.4	3.9	10.5
9	Amitriptyline	Basic	7.57	$C_{20}H_{23}N$	277.4	5.0	9.4

Recovery comparisons against competitor products

Recovery is an important metric in any sample preparation method. Higher recoveries allow more sensitive methods along with lower limits of quantification and detection. The data shown in *Figure 4* highlights how the Microlute™ CP Reversed Phase product can give very high recoveries for basic analytes.

Significantly higher recoveries of hydrophobic basic analytes can be seen when compared to competitor plates. For example, the recovery of amitriptyline for the Porvair Microlute™ product is 91.6%, comparing this value to the best performing competitor (competitor 3) at 69.6%. This shows an increase in recovery of 31.6%. An increase in recovery of 56.3% can be seen when comparing this recovery against the worst performing competitor.

It might be thought that such an increase in recoveries of more hydrophobic bases would result in a decrease in recoveries for the more hydrophilic bases. From the data collected, this is shown not to be the case. Instead there is a great balance in recoveries of both hydrophilic bases and hydrophobic bases. The Porvair Microlute™ CP RP maintains very high recoveries for the full range of basic compounds.

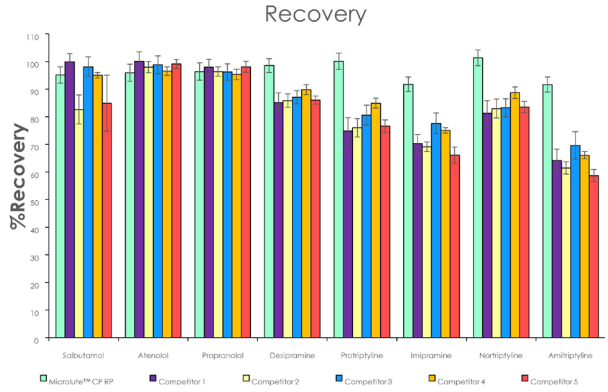

Figure 4. Basic analyte recovery comparisons against equivalent competitor SPE plates. Number of wells tested = 12. Error bars represent standard deviations.

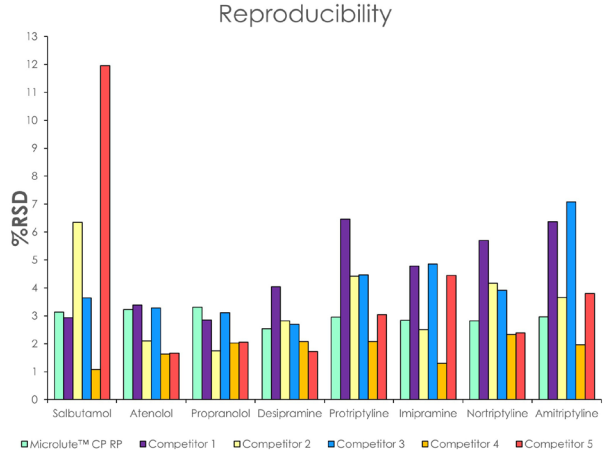

Figure 5. Basic analyte recovery %RSD comparisons against equivalent competitor SPE products. Number of wells tested = 12.

The basic analytes %RSD values can be seen *Figure 5*. The Porvair Microlute™ CP RP obtains %RSD values of less than 3.3% for every compound. This on average beats all competitors except competitor 4. However, when you consolidate both recovery and reproducibility metrics the Porvair Microlute™ CP RP combines better recoveries with great reproducibility values to produce the best all-around results for the recovery of basic compounds.

Conclusion

The Microlute™ CP RP 30 mg 96 well plate can selectively retain and elute basic compounds. The %RSD values are on average significantly lower than competitors, giving more reproducible results. The hybrid technology ensures even liquid flow rates throughout the SPE process, which leads to sufficient time for Van der Waals forces of interaction to take place between the sorbent and the analytes. No analyte is lost in the load step of the SPE process leading to high recovery values for all basic compounds. The Microlute™ CP RP 96 well-plate for solid phase extractions offers significant benefits for the recovery of hydrophobic basic analytes, as well as keeping %RSD values <5%.

References

1. *Poole, C., 2019. Solid-phase extraction. 1st ed. Elsevier, Chapter 3 - Porous polymer sorbents.*
2. *R. Veigure, K. Lossmann and M. Hecht et al. / Journal of Chromatography A 1613.*
3. *Pubchem. [online] Available at: PubChem (nih.gov)*