Efficient extraction of basic drugs from biological matrices using a polymeric cationic mixed-mode sorbent - strata™ X-C

Shahana Huq, Art Dixon, James Teuscher, Stanley Lok and Krishna Kallury
Phenomenex Inc., Torrance, CA, USA

Over 80% of drugs are classified as basic, which are characterized by the presence of an amine functional group that can accept a proton at low to neutral pH.[1] Direct analysis of these compounds in complex biomatrices such as urine or blood is often hampered by the presence of endogenous compounds in the sample. The interferences are often present in higher concentrations than that of the target analytes and thus may mask their presence. To circumvent this problem, solid phase extraction (SPE) is a sample preparation technique that is commonly used to purify and concentrate drug compounds and their metabolites prior to analysis.[2] In this application note, a simple and efficient method for the extraction of basic compounds from biological fluids using strata-X-C is presented.

strata-X-C is a revolutionary, patent pending polymeric resin that has been functionalized with polar and strong cation exchange groups. As a result, strata-X-C exhibits numerous retention mechanisms including hydrophobic, polar (i.e., hydrogen bonding), π-π, and strong cation exchange. Since the pKa of the strong cation exchange group is <1, the stationary phase is fully ionized (negatively charged) under aqueous conditions. In acidic solutions, the basic analyte will be ionized (positively charged) and thus can be retained by ionic interactions with the sorbent. Additional interactions (e.g., π-π, hydrophobic and hydrophilic) result from the polymeric backbone. The strong ionic retention mechanism allows the sorbent to be washed with relatively strong solvents, such as methanol, that can effectively remove anionic and neutral interferences without seriously affecting the recovery of the basic analyte. Finally, a mixture of organic solvent and ammonia is used to disrupt the analyte-sorbent interaction resulting in the elution of the basic compound.

Instrument and Equipment

Solid phase extraction

A 3mL syringe-barrel tube containing 60mg of polymer was used for the sample preparation of the target compounds. The physical and chemical properties of strata-X-C are listed in ***Table 1***. The SPE tubes were processed with a 12-position SPE vacuum manifold supplied by Phenomenex.

Liquid Chromatography

All analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with quaternary pump, in-line degasser, multi-wavelength detector and autosampler. HP Chemstation software was used to analyze the data. The HPLC column was a Synergi Max-RP, 4µm, 150 × 4.6 mm from Phenomenex.

Experimental Conditions

Specimen preparation

A urine or plasma sample (2-3mL) was spiked with a basic drug probe, ranging in concentration from 0.5-4.5µg/mL. The sample was then acidified with 20µL H_3PO_4/mL of sample and then diluted 1:1 using 100mM KH_2PO_4 buffer (pH 6).

Table 1. strata-X-C particle characteristics

Phase:	Surface modified styrene-divinylbenzene polymer
Average particle diameter:	33µm
Nominal pore size:	85Å
Surface area:	800m^2/g
Ionic capacity:	1meq/g
pH stability:	1-14

SPE method

Condition:	2mL methanol
Equilibrate:	2mL deionized H_2O
Load:	sample containing basic drug probe(s) at a flow rate of 1mL/min.
Wash 1:	2mL 0.1M HCl
Wash 2:	2mL methanol
Elution of basic compounds:	2mL 5% NH_4OH/methanol at a flow rate of 1mL/min.

Extraction Tips!

1. The solvent volumes shown above are for a 60mg bed mass. The solvent volumes will need to be optimized for smaller or larger bed masses.
2. Drying of the sorbent under vacuum after the first wash is optional. This is a significant advantage of strata-X-C as compared to conventional silica-based sorbents, which must be thoroughly dried prior to the organic wash step.
3. Any neutral and acidic compounds present may be fractionally collected with the organic wash step, if desired.

Analysis

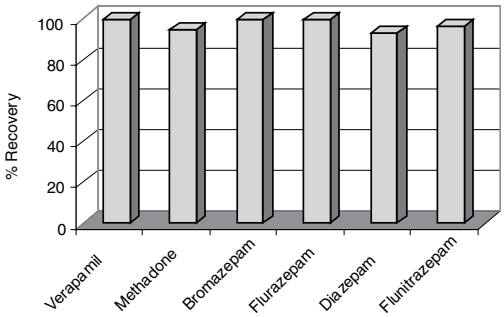
Drying: Extracts were dried at 40°C under a stream of nitrogen and reconstituted using the mobile phase buffer.

LC experimental conditions: 50-100µL of sample was injected. The specific conditions for each of the basic probes are given in Figure 1.

Results

Figure 1 shows the average recoveries of six basic drugs extracted from biological samples using strata-X-C. Verapamil and Methadone were extracted from urine (3mL). The other four compounds, belong to the class of compounds referred to as benzodiazepines, were extracted from plasma (2mL). The average recovery for each basic probe was greater than 90%. For all compounds, the RSD was ≤4%. This demonstrates that strata-X-C is effective in the extraction of basic compounds from biological matrices, with high and reproducible recoveries.

Figure 1. Average recoveries of basic drugs using strata-X-C

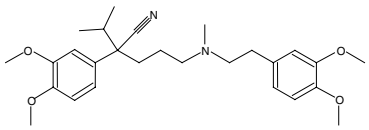


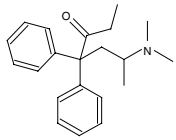
LC conditions:
For Verapamil: mobile phase consisted of A = 20mM KH_2PO_4 (pH 2.50), B = acetonitrile, C = methanol (62:25:13). The flow rate was 1mL/min with UV detection at 230nm.
For Methadone: mobile phase consisted of A = 0.1% TFA/water (pH 2.1), B = acetonitrile, (65:35). The flow rate was 1mL/min with UV detection at 210nm.
For the benzodiazepines: mobile phase consisted of A = 0.1% TFA/water (pH 2.1), B = 0.1% TFA/acetonitrile. The gradient was 5 to 95% B in 10 minutes. The flow rate was 1mL/min with UV detection at 254nm.

Conclusions

The innovative surface chemistry of the strata-X-C sorbent utilizes a strong ionic interaction between the sorbent and target basic analytes, allowing for aggressive washings with relatively strong organic solvents This results in superior clean up and concentration of the sample and low ion suppression under LC/MS conditions enabling error free quantitations.

This revolutionary polymeric technology exhibits significant advantages over the conventional silica-based SPE strong cation exchangers. First, the strata-X-C method gives high recoveries of basic compounds *without* the use of halogented elution solvents. In contrast, silica-based sorbents typically employ dichloromethane for analyte desorption. These harsh solvents require care when handling and have strict disposal requirements which increase the time and cost of sample preparation. Secondly, the rugged strata-X-C sorbent is resistant to deconditioning, making it very automation friendly. Lastly, the sorbent is stable over a wider pH range than silica-based sorbents, which dissolve under high pH conditions. These features make strata-X-C ideal for the extraction and clean up of low concentrations of basic drugs from very complex biological matrices.


Verapamil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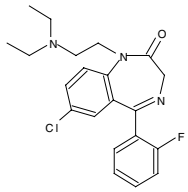
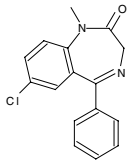

Flurazepam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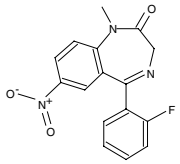
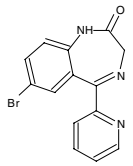

Flunitrazepam


Bromazepam

References

1. *Solid Phase Extraction Principles and Practice* by Thurman and Mills, John Wiley & Sons, 1998, see Chapter 8.
2. *Solid Phase Extraction Principles and Practice*, *Techniques and Applications* edited by Nigel Simpson, Marcel Dekker, Inc. 2000, see Chapters 7-10.

Acknowledgments

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Ordering Information

Order No.	Description
8B-S029-TAK-TN	strata-X-C 30mg/1mL Tubes (100/Box)
8B-S029-UBJ-TN	strata-X-C 60mg/3mL Tubes (50/Box)
8B-S029-FBJ-TN	strata-X-C 200mg/3mL Tubes (50/Box)
8B-S029-HBJ-TN	strata-X-C 500mg/3mL Tubes (50/Box)
8B-S029-ECH-TN	strata-X-C 100mg/6mL Tubes (30/Box)
8B-S029-FCH-TN	strata-X-C 200mg/6mL Tubes (30/Box)
8B-S029-HCH-TN	strata-X-C 500mg/6mL Tubes (30/Box)
8B-S029-EDG-TN	strata-X-C 100mg/12mL GigaTubes (20/Box)
8B-S029-HDG-TN	strata-X-C 500mg/12mL Giga Tubes (20/Box)
8B-S029-JDG-TN	strata-X-C 1000mg/12mL GigaTubes (20/Box)
8E-S029-AGB-TN	strata-X-C 96-Well Plate 10mg/well (2/Box)
8E-S029-TGB-TN	strata-X-C 96-Well Plate 30mg/well (2/Box)
00F-4337-E0-TN	Synergi Max-RP, 4µm, 150 x 4.6mm
AH0-6023-TN	12-Position SPE Vacuum Manifold

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