



Automated Solid Phase Extraction of Acid, Basic and Neutral Drugs in Animal Urine

Introduction

Routine analysis of human urine is well documented and straightforward but some laboratories have to test horses, greyhounds, bulls and camels. These animals have more viscous urine with more interferences so the samples need to be diluted in phosphate buffer pH6 prior to clean up. The protein also needs to be precipitated prior to testing which is common for all analysis. With human samples it is usual for labs to screen for a particular class of drug like opiates or THC. When dealing with animals the history is not so clear so a broad screen of acidic, basic and neutral drugs is required.

Instrumentation Used for Sample Preparation



The RapidTrace for Automated SPE



The TurboVap LV for Evaporation

Sample Analysis

A suitable sample volume (approximately 4 mL urine + 4 mL buffer) should be put in a 13 mm x 100 mm sample test tube ready for the clean up. The ideal SPE column is a 3 mL, HCX 300 mg SPE Isolute cartridge or equivalent. This is a mixed mode phase which has a C18 Silica support to capture neutral and acidic molecules and a cationic exchange phase to retain basic drugs. All concentration steps that require evaporation, especially the Eluent, can be carried out quickly using the 50 sample TurboVap LV with a 12 mm x 75 mm test tube rack. The method collects an acidic and neutral fraction plus a second fraction, so 5 samples provide 10 fractions per instrument.

The RapidTrace Method

All solvent lines are first purged and primed with solvent. A maximum of 8 solvents can be used to run a wide range of methods. The 4 independent waste solvent lines can be separated if needed. The instrument sample rack has two rows of 10 test tubes, 13 mm x 100 mm for holding the samples and 12 mm x 75 mm for eluent fractions. This method offers an automated rugged and reproducible solution for cleaning up the samples to remove interferences.

Eluent 1 = Chloroform / Acetone (3:1), (Ethyl Acetate Hexane 25:75 can also be used).

Eluent 2 = DCM / Isopropanol/Ammonia (80:12:2) Ethyl Acetate can be used as an alternative to DCM.
Dissolve Ammonia in Isopropanol then add to DCM to prepare fresh each day.

Common Reagent Table for All Methods

Step	Source	Destination	Volume (mL)	Flow (mL/min)
Condition	Methanol	Organic Waste	2	15
Condition	Water	Aqueous Waste	2	15
Condition	Phosphate Buff	Aqueous Waste	2	15
Load	Sample	Organic Waste	6	1
Rinse	Phosphate Buff	Aqueous Waste	2	15
Rinse	Acetic Acid	Aqueous Waste	2	15
Purge-Cannula	Eluent 1	Cannula Waste	2	30
Dry	Nitrogen		5 minutes	
Collect	Eluent 1	Fraction 1	2	1
Rinse	Methanol	Organic Waste	2	15
Purge-Cannula	Eluent 2	Organic Waste	2	30
Dry	Nitrogen		5 minutes	
Collect	Eluent 2	Fraction 2	3	1
Purge-Cannula	Methanol	Cannula Waste	2	30
Purge-Cannula	Water	Cannula Waste	2	30

Line No	Reagent Name	Sip Speed (mL/min)	Waste Name	Abbreviation	
1	Methanol	30	Aqueous Waste	Aq W	Air Push = 2mL
2	Water	30	Organic Solvent	Org W	Air Push Multiplier = 2
3	Phosphate Buffer pH6	30	Cannula Waste	cannula	
4	Acetic Acid 1.0M	30	6	1	
5	Elute 1 (Acid / Neutral)	30	2	15	
6	Elute 2 (Basic Drugs)	30	2	15	

Summary

The Methanol is used to solvate the SPE phase and the weak acidic phosphate buffer 0.1 M pH 6.0 prepares the Cation exchange portion so that it can bind to the basic drugs. If a strong acid buffer (pH2.0) is used it will bind molecules too strongly and give poor recoveries. The C18 Phase captures non polar neutral organic drugs.

An Acetic Acid 1.0 M rinse is used to remove interferences. The non polar solvents can then elute the acidic and neutral fraction after drying the column. This is very important in order to obtain good recoveries and prevent double layers in the eluent.

A rinse with methanol, a poor organic solvent, will help remove further impurities without loss of basic drugs. It also helps hydrate the column for the drying step. The second eluent which should have a pH10 is designed to change the PKa value to allow total removal of the basic drugs held by the cationic exchange phase. The cannula wash steps prevent any carry over between samples.

Acknowledgements

This application note has been prepared to help users of the RapidTrace and TurboVap get started with a drugs method and has been based on experience with customers doing this analysis with Caliper Life Sciences instrumentation.



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