

## SEP-PAK® C<sub>18</sub> CARTRIDGE SAMPLE CLEANUP FOR QUANTITATION OF SEROTONIN IN HUMAN PLASMA, SERUM AND CEREBROSPINAL FLUID BY LC

The author (1) reports using 6-hydroxytryptamine (6-HT) as an internal standard for quantitation of 5-hydroxytryptamine (serotonin, 5-HT), and using SEP-PAK C<sub>18</sub> cartridges as one method for handling the cleanup of biological samples where recovery and concentration of low levels are important issues.

A GC/MS was utilized to validate the LC-EC methods used following sample cleanup procedures. Standard calibration curves were generated by plotting 5-HT/6-HT ratios against 5-HT concentrations.

### SEP-PAK® Sample Cleanup

Plasma: To 1 ml of plasma was added 200 ng 6-HT and the mixture made alkaline with 0.5 ml 10% sodium carbonate. The mixture was passed through a SEP-PAK® C<sub>18</sub> cartridge which had been activated prior to use by washing with 2 ml of methanol followed with 2 ml distilled water. The sample was washed on the cartridge with 1 ml of water and then eluted with 4 ml methanolic formic acid (90 ml methanol + 10 ml formic acid). The eluate was then evaporated under nitrogen and the residue was redissolved in 0.2 ml mobile phase. A 50 µl injection was made on the LC-EC (electrochemical detection).

Platelet Samples: Blood samples were centrifuged to obtain a platelet count of 500,000 to 600,000/µl. A 0.5 ml sample was combined with 0.5 ml of mobile phase and 500 ng of 6-HT (internal standard). The mixture was sonicated for 30 seconds and 25 µl was injected directly on the LC-EC. One half the remaining volume was combined with 0.5 ml 10% sodium carbonate and processed in a SEP-PAK® C<sub>18</sub> cartridge as above.

Cerebrospinal Fluid: A 2 ml sample of cerebrospinal fluid was spiked with 5 ng 6-HT and made alkaline with 0.5 ml 10% sodium carbonate and applied to a SEP-PAK® C<sub>18</sub> cartridge. The loaded cartridge was then washed with 1 ml of hexane followed by 2 ml of methanolic formic acid. The eluate was evaporated at 40°C under nitrogen and the residue redissolved in 0.1 ml mobile phase and 40 µl injected on the LC-EC.

The sample cleanup was reported as good. Table 1 shows a comparison of the method against a direct injection. Table 2 shows serotonin levels in cerebrospinal fluid. The author presently gives a detection limit of 200 pg of serotonin per ml cerebrospinal fluid.

The author emphasizes this sample cleanup method to greatly improve the efficiency of recovery over solvent extraction methods. Also, this method enables the concentration of the extremely low levels (1 ng/ml) of serotonin in cerebrospinal fluid.

TABLE 1

Serotonin Levels in Plasma, Serum and Platelet-rich Plasma  
Samples. ng/ml

Sample	SEP-PAK	Direct Injection
Plasma (MAOI)	730	
Plasma (MAOI)	500	
Serum pooled (N=4)		
PRP	960	970
PRP	525	510
PRP	728	760

TABLE 2

Serotonin Levels in Human CSF

Sample	5HT(ng/ml)	Method
1	0.81	SEP-PAK C <sub>18</sub>
2	1.25	SEP-PAK C <sub>18</sub>
Pooled CSF (N=4)	1.25±0.15	SEP-PAK

Standard Calibration Curve  $r = 0.992$

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THE PERFORMANCE CHARACTERISTIC FOR THIS  
PROCEDURE HAS NOT BEEN ESTABLISHED.