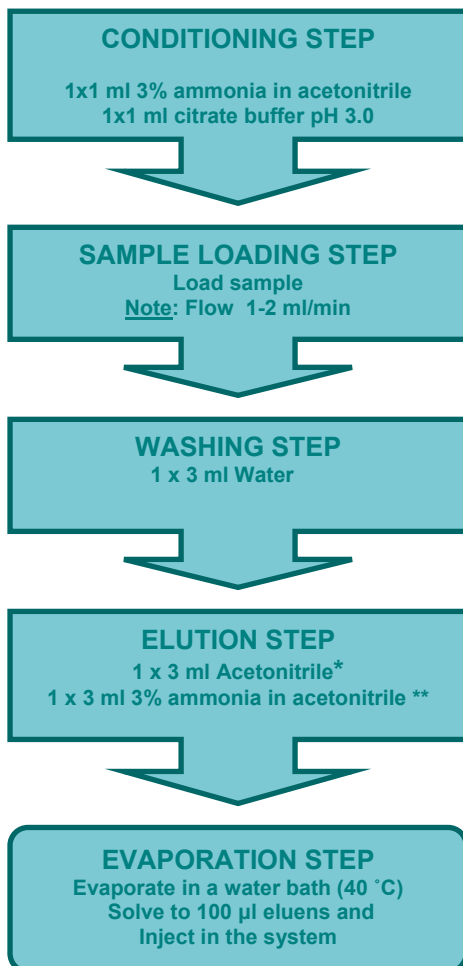


Systematic Toxicological Identification Procedure (STIP) as a TDM-method for Clozapine, D-Clozapine, Nortriptyline, Hydroxynortriptyline and Amitriptyline in serum after Solid Phase Extraction V2009

EXTRACTION PROTOCOL



SAMPLE MATRIX Serum

SORBENT BAKERBOND™ *Speedisk C₁₈*
Polar Plus 3ml/50 mg; PN 8153-04

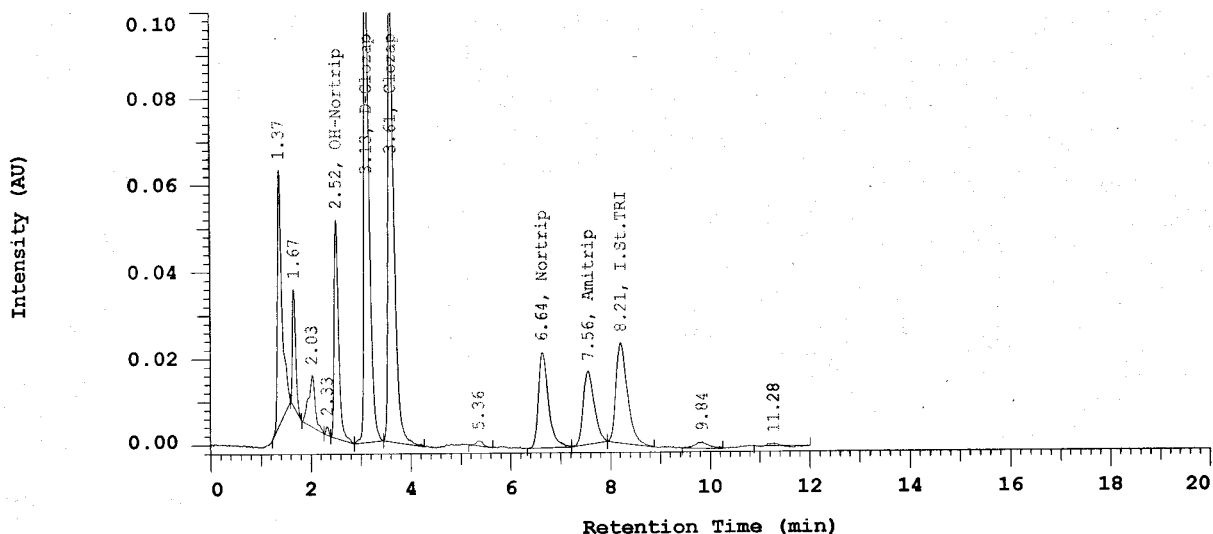
SAMPLE PREPARATION

To 1 ml of serum add 2 ml of internal standard solution of trimipramine. Internal standard solution is made by dissolving appropriate amount of trimipramine in citrate buffer pH 3.0 to obtain the concentration of 0.1 µg/ml of trimipramine. The sample is applied to the spe column.

- * A₁ fraction - elution of acidic and neutral compounds – vial 1
- ** A₂ fraction - elution of basic compounds – vial 2

FINAL ANALYTICAL METHOD: HPLC-DAD According to STIP

Chrom Type: Fixed WL Chromatogram, 205 nm



Peak Quantitation: HEIGHT

Calculation Method: INT-STD

RT	Area	Height	Purity	ID-Cor	Name	Conc 1 ug/L
2.52	152390	25250	0.9999		OH-Nortrip	100.000
3.13	422260	58079	0.9998		D-Clozap	500.000
3.61	440350	52642	0.9998		Clozap	511.900
6.64	155780	11122	0.9998		Nortrip	99.7100
7.56	130380	8499	0.9996		Amitrip	100.600
8.21	189420	11657	0.9998		I.St.TRI I	

Validation/storage life:

	ug/l	Linearity	Accuracy/Precision: Standard ug/l	Within-Run / Between-Run Cor. of Var %	
OH-Nortriptyline	37.5-300	0.9991	150	2.3 /	3.1
D-Clozapine	62.5-500	0.9939	250	2.2 /	1.3
Clozapine	250-2000	0.9985	1000	3.7 /	2.1
Nortriptyline	37.5-300	0.9992	150	2.4 /	3.9
Amitriptyline	37.5-300	0.9997	150	1.3 /	0.0

	Recovery	Storage life Control serum -20°C
OH-Nortriptyline	>90%	: min. 1 year
D-Clozapine	>90%	: min. 1 year
Clozapine	>90%	: min. 1 year
Nortriptyline	>90%	: min. 1 year
Amitriptyline	>90%	: min. 1 year

For more information on STIP system and Solid Phase Extraction Procedure for antidepressants and antipsychotic drugs visit www.zanob.nl

For more info on Solid Phase Extraction Products and application database, visit www.jtbaker.com