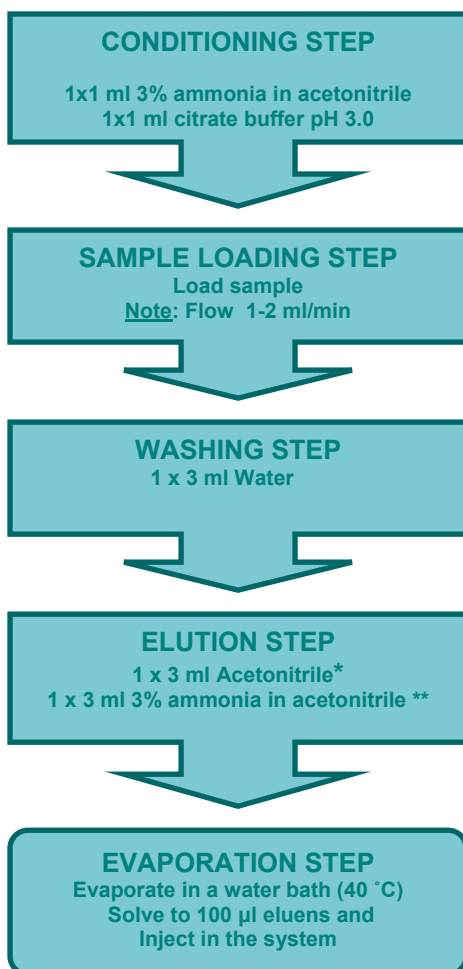


Systematic Toxicological Identification Procedure (STIP) as a TDM-method for Desipramine, Imipramine, Clomipramine and D-Clomipramine in serum after Solid Phase Extraction

V2009

EXTRACTION PROTOCOL



SAMPLE MATRIX Serum

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

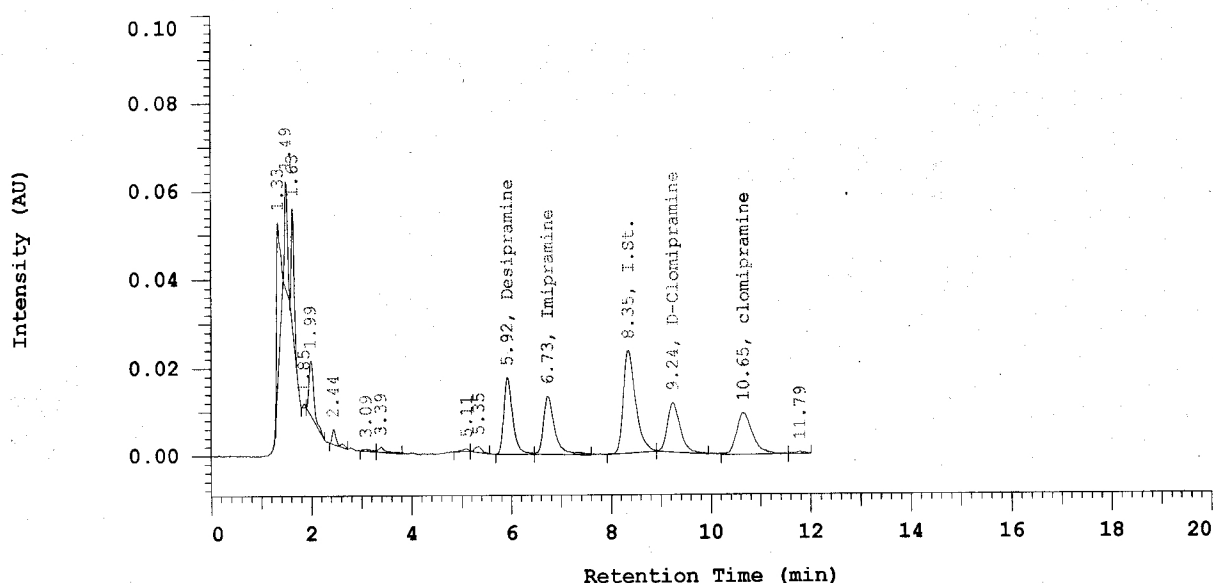
SAMPLE PREPARATION

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 |
|-------|--------|--------|--------|--------|----------------|----------------|
| 5.92 | 109570 | 8792 | 0.9994 | | Desipramine | 100.000 |
| 6.73 | 104870 | 6654 | 0.9997 | | Imipramine | 105.500 |
| 8.35 | 202340 | 11688 | 0.9998 | | I.St. | I |
| 9.24 | 101120 | 5687 | 0.9994 | | D-Clomipramine | 98.2200 |
| 10.65 | 106410 | 4791 | 0.9992 | | clomipramine | 102.100 |

Validation/storage life:

| | ug/l | Linearity | Accuracy/Precision: Standard ug/l | Within-Run / Between-Run Cor. of Var % |
|----------------|----------|-----------|--------------------------------------|---|
| Desipramine | 37.5-300 | 0.9984 | 150 | 5.4 / 0.0 |
| Imipramine | 37.5-300 | 0.9985 | 150 | 5.1 / 2.0 |
| D-Clomipramine | 37.5-300 | 0.9985 | 150 | 5.3 / 0.0 |
| Clomipramine | 37.5-300 | 0.9961 | 150 | 5.5 / 3.5 |

| | Recovery | Storage life Control serum -20°C |
|----------------|----------|----------------------------------|
| Desipramine | >90% | : min. 1 year |
| Imipramine | >90% | : min. 1 year |
| D-Clomipramine | >90% | : min. 1 year |
| Clomipramine | >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