# Antidepressants in urine using the Triage® TOX Drug Screen fluorescent immunoassay followed by confirmation via direct injection LC-MS/MS

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## Introduction

Tricyclic antidepressants (TCA) are used for the treatment of anxiety disorders, mild to moderate pain as well as depression; however there are serious negative side-effects associated with their routine use, including dizziness, drowsiness, increased heart rate and even death. In suspected toxic cases, the availability of a rapid test is useful, but previous TCA immunoassays have suffered from poor specificity and/or false positive results.

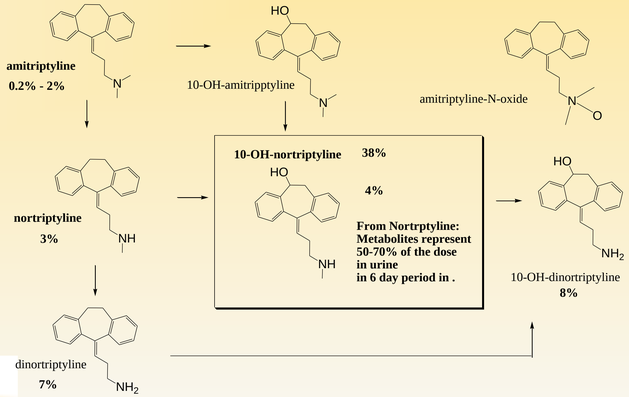
## Objectives

To determine the contribution of glucuronidated urine metabolites towards a positive result using an immunoassay. Since glucuronide reference compounds are not commercially available, the identification of antidepressant metabolites in urine was carried out by LC-MS/MS before and after hydrolysis of the authentic specimens to characterize the immunoreactive assay.


Urine

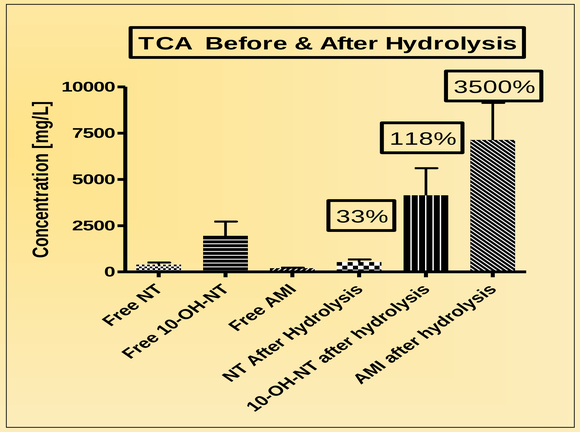
## Methods

Urine specimens were screened for the presence of TCAs using a fluorescent immunoassay (Alere Triage® TOX Drug Screen) calibrated with desipramine at a cut-off 1000 mg/L. The positive specimens were analyzed for parent TCA drugs using a standard LC/MS/MS method consisting of an Agilent 1200 Series HPLC equipped with a Zorbax Eclipse XDB C18 (4.6 x 50mm x 1.8 mm) heated to 45°C, coupled to a 6410 triple quadrupole mass spectrometer, operating in positive electrospray ionization mode (ESI). Samples were eluted using a gradient of methanol and water with 0.2% acetic acid. A new LC/MS/MS method was developed for the identification and quantification of hydroxylated and demethylated TCA metabolites. In addition, using this new method, the same specimens were analyzed before and after hydrolysis with β-glucuronidase to assess glucuronide contributions to the positive results in the immunoassay.




## Results & Discussion

While the concentration of the measured antidepressants increased following hydrolysis, the most dramatic increases were for amitriptyline (AMI). 7 samples contained nortriptyline (NT), 10-OH-NT & AMI. Following hydrolysis, NT averaged an increase of 33%; 10-OH-NT, 118% & AMI over 3500%.




**TCA Method Comparison: TOX DS vs. TOX DS II;**
Positive (N=48) % Agreement 100 (95%CI) (92.3,100.0)
Negative (N=40) % Agreement 1000 (95%CI) (91.2,100.0)
**Total % Agreement (95% CI) 100 (95.8,100.0)**

Note: All 95% Confidence intervals were calculated using the Wilson Score with no continuity correction applied.

## Summary

Quantitation of parent TCAs and their hydroxylated and demethylated metabolites by LC/MS/MS is insufficient when correlating with immunoassay based screening protocols like Triage® TOX Drug Screen, which also detects glucuronidated TCA metabolites. LC/MS/MS results generated after enzymatic hydrolysis of TCA metabolite glucuronides account for total amounts of TCAs and their metabolites, and such results correlate well with Triage® TOX Drug Screen results.

## References

1. C. Coulter, M. Taruc, J. Tuyay, and C. Moore. Antidepresant Drugs in Oral Fluid Using Chromatography-Tandem Spectrometry. *J. Anal. Toxicol.* **34:** 1-9 (2010).
2. Stacy E.F. Melanson, E. Lee Lewandowski, David A. Griggs, and James G. Flood. Interpreting Tricyclic Antidepresant Measurements in Urine in an Emergency Department Setting: Comparison of Two Qualitative Point-of-Care Urine Tricyclic Antidepresant Drug Immunoassay with Quantitative Serum Chromatographic Analysis. *J. Anal. Toxicol*. **31:** 270-275 (2007).
3. Product Inserts: Alere Triage® TOX Drug Screen (DS) has the following assays
APAP, AMP, mAMP, BAR, BZO, COC, MTD, OPI, PCP , THC; Alere Triage® TOX Drug Screen II (510k submitted to FDA) has TOX DS menu plus SAL, MDMA, and OXY assays.

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