TECHNICAL NOTE 66052

# Quantification of 15 tricyclic antidepressants in human plasma by liquid chromatography-tandem mass spectrometry for clinical research

Authors: Gaëtan Renoulin,[1] Mariana Barcenas,[1] and Claudio De Nardi[2]
[1]Thermo Fisher Scientific, Les Ulis, France
[2]Thermo Fisher Scientific, Reinach, Switzerland

Keywords: TraceFinder software, TSQ Quantis MS, Vanquish Flex UHPLC, LC-MS, mass spectrometry, offline sample preparation, plasma, tricyclic antidepressants

## Application benefits

- Robust, sensitive hardware enables increased confidence in data
- Simple offline sample preparation by protein precipitation
- Fifteen tricyclic antidepressants in a single quantitative method

## Goal

Implementation of an analytical method for the quantification of 15 tricyclic antidepressants in human plasma on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer.

## Introduction

Tricyclic antidepressants (TCAs) are a group of psychoactive drugs that are mainly used to alleviate symptoms of anxiety, endogenous depression, and pain.


In this report, an analytical method for clinical research for the quantification of 15 tricyclic antidepressants in human plasma in eight minutes is presented. The samples were prepared offline by protein precipitation. Extracted samples were chromatographically separated on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system. Detection was performed on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization (HESI) operated in positive ionization mode. Method performance was evaluated using the ClinMass® Add-on Set for TCAs, including LC-MS/MS calibrators, controls, and internal standards from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response, lower limit of quantification (LLOQ), accuracy, and intra- and inter-assay precision for all analytes.

Table 1. List of analytes, ranges of concentrations (MS9113 Batch #1389), and internal standards

| Compound name | Formula | Concentration Range (µg/L) | Internal standard name | Formula |
|---|---|---|---|---|
| Amitriptyline | $C_{20}H_{23}N$ | 16.3–331 | Amitriptyline-$d_3$ | $C_{20}H_{20}D_3N$ |
| Clomipramine | $C_{19}H_{23}ClN_2$ | 18.3–361 | Clomipramine-$d_3$ | $C_{19}H_{20}D_3ClN_2$ |
| Clozapine | $C_{18}H_{19}ClN_4$ | 58.5–1202 | Clozapine-$d_4$ | $C_{18}H15D_4ClN_4$ |
| Desipramine | $C_{18}H_{22}N_2$ | 18.1–377 | Desipramine-$d_3$ | $C_{18}H_{19}D3N_2$ |
| Doxepin | $C_{19}H_{21}NO$ | 14.8–287 | Doxepin-$d_3$ | $C_{19}H_{18}D_3NO$ |
| Imipramine | $C_{19}H_{24}N_2$ | 16.7–343 | Imipramine-$d_3$ | $C_{19}H_{21}D_3N_2$ |
| Maprotiline | $C_{20}H_{23}N$ | 24.2–465 | Maprotiline-$d_5$ | $C_{20}H_{20}D_3N$ |
| Norclomipramine | $C_{18}H_{21}ClN_2$ | 21.1–416 | Norclomipramine-$d_3$ | $C_{18}H_{18}D_3ClN_2$ |
| Norclozapine | $C_{17}H_{17}ClN_4$ | 47.3–950 | Norclozapine-$d_8$ | $C_{17}H_9D_8ClN_4$ |
| Nordoxepin | $C_{18}H_{19}NO$ | 13.6–279 | Nordoxepin-$d_3$ | $C_{18}H_{16}D_3NO$ |
| Normaprotiline | $C_{19}H_{21}N$ | 33.4–725 | Desipramine-$d_3$ | $C_{18}H_{19}D_3N_2$ |
| Nortrimipramine | $C_{19}H_{24}N_2$ | 11.0–220 | Imipramine-$d_3$ | $C_{19}H_{21}D_3N_2$ |
| Nortriptyline | $C_{19}H_{21}N$ | 18.2–371 | Nortriptyline-$d_3$ | $C_{19}H_{18}D_3N$ |
| Protriptyline | $C_{19}H_{21}N$ | 16.9–344 | Nortriptyline-$d_3$ | $C_{19}H_{18}D_3N$ |
| Trimipramine | $C_{20}H_{26}N_2$ | 27.3–579 | Trimipramine-$d_3$ | $C_{20}H_{23}D_3N_2$ |

## Experimental

### Target analytes

The complete list of analytes with their corresponding internal standards and the concentration ranges covered by the calibrators used are reported in Table 1.

### Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE, as well as an internal standard mix for quantitation. Samples of 50 µL of plasma were protein precipitated using 100 µL of acetonitrile containing the internal standards. Precipitated samples were vortex-mixed and centrifuged for 10 minutes. The supernatant was transferred to a clean vial.

### Liquid chromatography

The supernatant was injected onto a Vanquish Flex Binary UHPLC system connected to a TSQ Quantis triple quadrupole mass spectrometer. Chromatographic separation was achieved by gradient elution on a Thermo Scientific™ Hypersil GOLD™ Phenyl (100 × 2.1 mm, 1,9 µm) column (P/N 25902-102130) kept at 40 °C.

Table 2. LC gradient profile

| Time | Flow (mL/min) | %B |
|---|---|---|
| 0.00 | 0.500 | 10.00 |
| 0.20 | 0.500 | 10.00 |
| 0.50 | 0.500 | 50.00 |
| 4.50 | 0.500 | 50.00 |
| 5.20 | 0.500 | 100.0 |
| 6.50 | 0.500 | 100.0 |
| 6.51 | 0.500 | 10.00 |
| 8.00 | 0.500 | 10.00 |

Mobile phases composition is the following:

- Mobile phase A: Water + 0.1% formic acid + 5 mM ammonium formate
- Mobile phase B: Methanol + 0.1% formic acid + 5 mM ammonium formate

Injection volume was 2 µL.

The LC gradient is described in Table 2. Total runtime was 8.0 minutes.

## Mass spectrometry

Analytes and internal standards were detected by selected reaction monitoring (SRM) on a TSQ Quantis triple quadrupole mass spectrometer using a HESI source operated in positive ionization mode. A summary of the MS conditions is reported in Table 3. One confirming ion for each analyte was included in the acquisition method for confirmation (Table 4).

## Method evaluation

The performance of the method was evaluated in terms of carryover, LLOQ, accuracy, and intra- and inter-assay precision for all analytes.

Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected just after it.

Table 3. MS parameters

| Ion source parameters | |
|---|---|
| Source type | Heated Electrospray Source Ionization (HESI) |
| Spray voltage – Positive (V) | 3,500 |
| Sheath gas (Arb) | 50 |
| Aux gas (Arb) | 10 |
| Sweep gas (Arb) | 0 |
| Ion transfer tube temp. (°C) | 300 |
| Vaporizer temp. (°C) | 320 |
| **Settings** | |
| Data acquisition mode | SRM |
| **SRM parameters** | |
| Cycle time (s) | 0.3 |
| Q1 resolution (FWHM) | 0.7 |
| Q3 resolution (FWHM) | 1.2 |
| Chromatographic peak width (s) | 6 |

Table 4. Description of the SRM parameters

| Analyte/Internal standard | Retention (min) | Quantification | | | Confirmation | | | RF lens (V) |
|---|---|---|---|---|---|---|---|---|
| | | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (V) | Precursor ion (*m/z*) | Product ion (*m/z*) | Collision energy (V) | |
| Amitriptyline | 5.6 | 278.2 | 105.1 | 23 | 278.2 | 233.2 | 17 | 122 |
| Clomipramine | 5.9 | 315.2 | 86.2 | 18 | 315.2 | 58.13 | 29 | 119 |
| Clozapine | 3.3 | 327.1 | 192.1 | 44 | 327.1 | 270.1 | 22 | 148 |
| $d_3$-Amitriptyline | 5.6 | 281.2 | 281.2 | 23 | 281.2 | — | — | 122 |
| $d_3$-Clomipramine | 5.9 | 318.2 | 318.2 | 18 | 318.2 | — | — | 119 |
| $d_3$-Desipramine | 5.1 | 270.2 | 270.2 | 16 | 270.2 | — | — | 101 |
| $d_3$-Doxepin | 3.4 | 283.2 | 283.2 | 23 | 283.2 | — | — | 123 |
| $d_3$-Imipramine | 5.1 | 284.2 | 284.2 | 28 | 284.2 | — | — | 111 |
| $d_3$-Norclomipramine | 5.9 | 304.2 | 304.2 | 17 | 304.2 | — | — | 112 |
| $d_3$-Nordoxepin | 3.4 | 269.2 | 269.2 | 22 | 269.2 | — | — | 117 |
| $d_3$-Nortriptyline | 5.6 | 267.2 | 267.2 | 14 | 267.2 | — | — | 111 |
| $d_3$-Trimipramine | 5.7 | 298.2 | 298.2 | 17 | 298.2 | — | — | 111 |
| $d_4$-Clozapine | 3.3 | 331.2 | 331.2 | 44 | 331.2 | — | — | 148 |
| $d_5$-Maprotiline | 5.4 | 283.2 | 283.2 | 19 | 283.2 | — | — | 128 |
| $d_8$-Norclozapine | 3.2 | 321.2 | 321.2 | 41 | 321.2 | — | — | 157 |
| Desipramine | 5.1 | 267.2 | 72.13 | 16 | 267.2 | 44.07 | 42 | 101 |
| Doxepin | 3.4 | 280.2 | 107 | 23 | 280.2 | 58.13 | 21 | 123 |
| Imipramine | 5.1 | 281.2 | 58.13 | 30 | 281.2 | 86.2 | 17 | 107 |
| Maprotiline | 5.4 | 278.2 | 250.2 | 25 | 278.2 | 178.1 | 39 | 128 |
| Norclomipramine | 5.9 | 301.2 | 72.13 | 17 | 301.2 | 44.07 | 49 | 112 |
| Norclozapine | 3.2 | 313.1 | 192.13 | 41 | 313.1 | 270.1 | 24 | 157 |
| Nordoxepin | 3.4 | 266.2 | 107.1 | 22 | 266.2 | 44.07 | 18 | 117 |
| Normaprotiline | 5.3 | 264.2 | 169.2 | 18 | 264.2 | 219.1 | 22 | 113 |
| Nortrimipramine | 5.7 | 281.2 | 44.07 | 28 | 281.2 | 55.05 | 30 | 108 |
| Nortriptyline | 5.6 | 264.2 | 233.2 | 14 | 264.2 | 105.1 | 20 | 111 |
| Protriptyline | 5.0 | 264.2 | 233.2 | 16 | 264.2 | 155.1 | 21 | 125 |
| Trimipramine | 5.7 | 295.2 | 100.1 | 17 | 295.2 | 58.13 | 28 | 111 |

Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at two different levels provided by RECIPE (MS9182 batch #1389), prepared and analyzed in replicates of five on three different days. Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

LLOQ was investigated by dilution of the lowest calibrator using blank matrix and was established as the lowest concentration with a mean accuracy and precision below than 20%.

### Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 5.1 software.

## Results and discussion

A linear interpolation with 1/x weighting was used for all analytes. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators in all the runs. Representative chromatograms at the LLOQ for clozapine, norclomipramine, nortriptyline, trimipramine, and their corresponding internal standards are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

No carryover was observed for any of the analytes, with no signal detected in the blank injected immediately after the highest calibrator.

The data demonstrated good accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -8.6% and 9.3% (Table 5). The %CV for intra-assay precision was always below 6.2% for all the analytes. The maximum %CV for inter-assay precision including all the analytes was 6.2%. Results for intra- and inter-assay precision are reported in Table 6.

LLOQs of all compounds were determined and reported in Table 7.

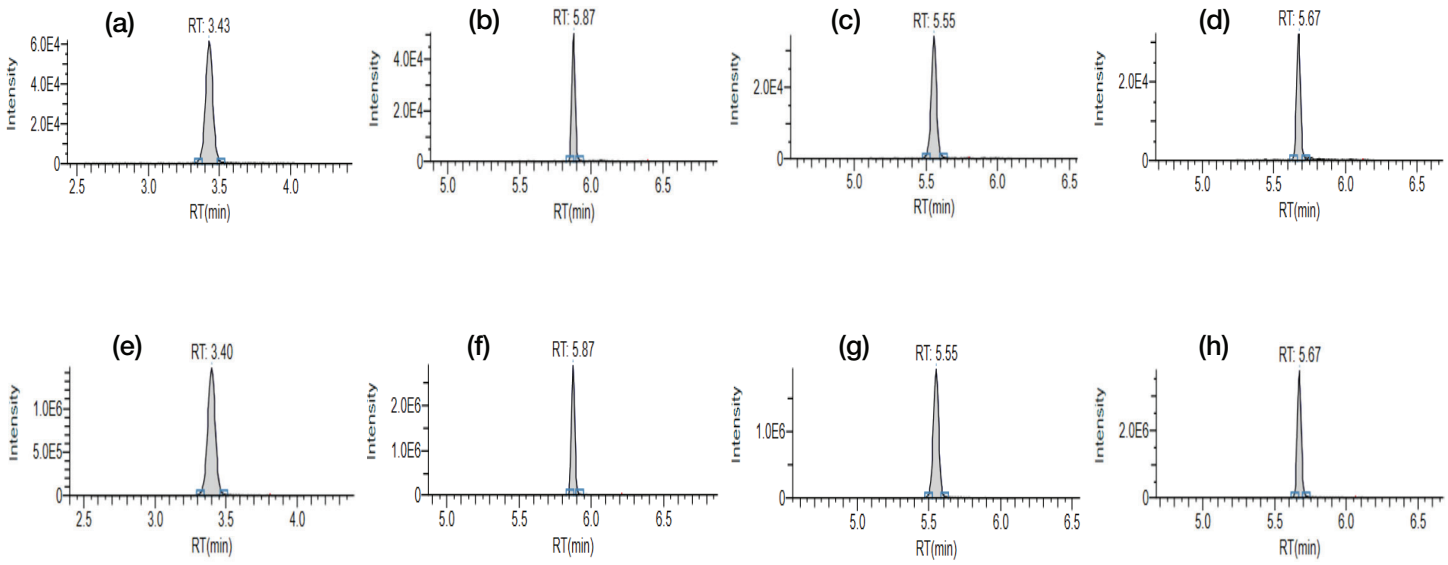


**Figure 1. Representative chromatograms of the lower limits of quantification for (a) clozapine, (b) norclomipramine, (c) nortriptyline, (d) trimipramine, (e) $d_4$-clozapine, (f) $d_3$-norclomipramine, (g) $d_3$-nortriptyline, (h) $d_3$-trimipramine**

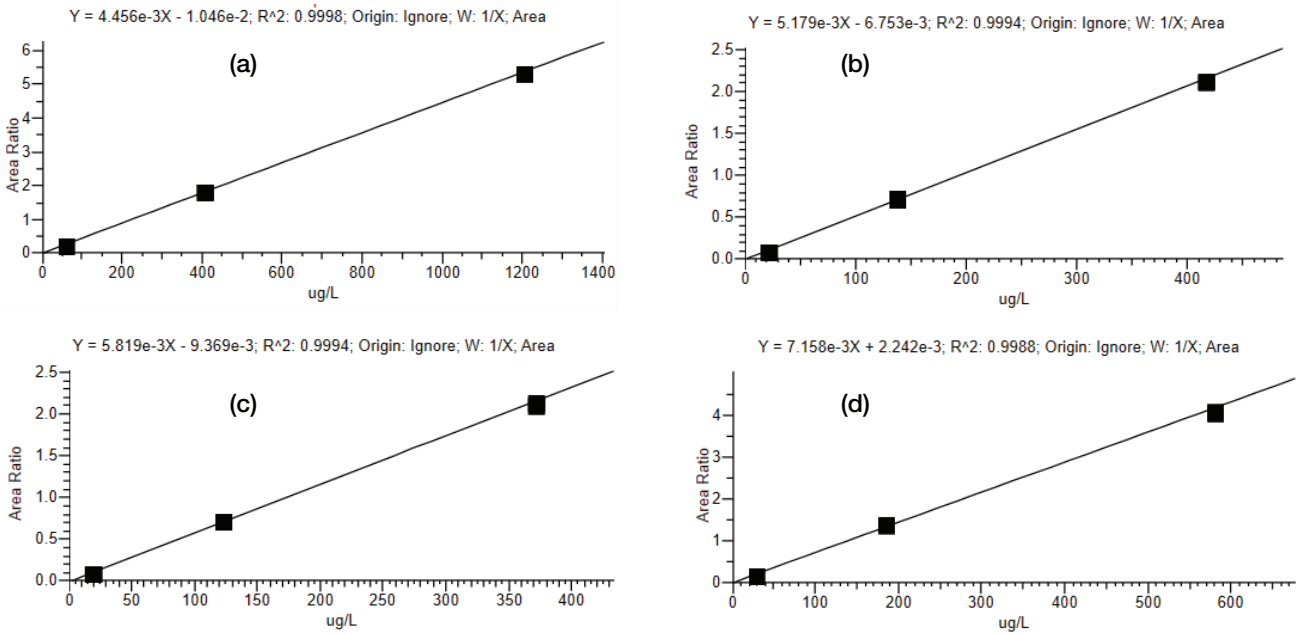


Figure 2. Representative calibration curves for (a) clozapine, (b) norclomipramine, (c) nortriptyline, (d) trimipramine

Table 5. Analytical accuracy results for control MS9182 Batch #1389

| Analyte | Control | Nominal concentration (µg/L) | Average calculated concentration (µg/L) | Bias (%) |
|---|---|---|---|---|
| Amitriptyline | Level I | 59.1 | 60.9 | 3.1 |
| | Level II | 137 | 149 | 8.8 |
| Clomipramine | Level I | 67.3 | 69.3 | 3.0 |
| | Level II | 161 | 165 | 2.7 |
| Clozapine | Level I | 229 | 228 | -0.6 |
| | Level II | 529 | 540 | 2.1 |
| Desipramine | Level I | 68.1 | 66.9 | -1.7 |
| | Level II | 158 | 164 | 3.6 |
| Doxepin | Level I | 53.3 | 52.3 | -1.9 |
| | Level II | 123 | 128 | 4.3 |
| Imipramine | Level I | 62.2 | 61.0 | -1.9 |
| | Level II | 143 | 152 | 6.1 |
| Maprotiline | Level I | 86.6 | 83.0 | -4.2 |
| | Level II | 199 | 200 | 0.4 |
| Norclomipramine | Level I | 78.7 | 77.3 | -1.7 |
| | Level II | 180 | 183 | 1.9 |
| Norclozapine | Level I | 183 | 180 | -1.5 |
| | Level II | 413 | 422 | 2.2 |
| Nordoxepin | Level I | 50.6 | 49.1 | -3.0 |
| | Level II | 118 | 121 | 2.5 |
| Normaprotiline | Level I | 129 | 118 | -8.6 |
| | Level II | 297 | 295 | -0.8 |
| Nortrimipramine | Level I | 40.4 | 39.9 | -1.2 |
| | Level II | 90.8 | 98.2 | 8.1 |
| Nortriptyline | Level I | 69.1 | 69.0 | -0.2 |
| | Level II | 160 | 166 | 3.7 |
| Protriptyline | Level I | 61.8 | 60.2 | -2.5 |
| | Level II | 146 | 147 | 0.9 |
| Trimipramine | Level I | 102 | 108 | 6.2 |
| | Level II | 240 | 262 | 9.3 |

Table 6. Analytical intra- and inter-assay precision results for control MS9182 Batch #1389

| Analyte | Control | Intra-assay | | | | | | Inter-assay | |
|---|---|---|---|---|---|---|---|---|---|
| | | Day 1 | | Day 2 | | Day 3 | | | |
| | | Average calculated concentration (µg/L) | CV (%) | Average calculated concentration (µg/L) | CV (%) | Average calculated concentration (µg/L) | CV (%) | Average calculated concentration (µg/L) | CV (%) |
| Amitriptyline | Level I | 57.8 | 2.5 | 62.6 | 2.7 | 62.5 | 4.0 | 60.9 | 4.5 |
| | Level II | 141 | 4.8 | 153 | 5.5 | 153 | 3.6 | 149 | 4.5 |
| Clomipramine | Level I | 66.2 | 2.2 | 70.7 | 3.5 | 71.0 | 2.9 | 69.3 | 3.8 |
| | Level II | 157 | 4.7 | 171 | 4.4 | 168 | 4.3 | 165 | 4.6 |
| Clozapine | Level I | 220 | 2.3 | 231 | 3.5 | 232 | 2.9 | 227 | 2.8 |
| | Level II | 523 | 4.4 | 554 | 4.0 | 543 | 4.6 | 540 | 2.9 |
| Desipramine | Level I | 64.7 | 1.9 | 68.9 | 4.0 | 67.1 | 2.7 | 66.9 | 3.1 |
| | Level II | 158 | 5.3 | 171 | 4.8 | 163 | 3.6 | 164 | 4.0 |
| Doxepin | Level I | 50.5 | 3.1 | 52.7 | 3.1 | 53.7 | 2.6 | 52.3 | 3.1 |
| | Level II | 123 | 4.7 | 131 | 5.2 | 130 | 4.4 | 128 | 3.2 |
| Imipramine | Level I | 58.6 | 2.8 | 62.3 | 4.6 | 62.1 | 2.6 | 61.0 | 3.5 |
| | Level II | 145 | 5.1 | 156 | 4.6 | 154 | 4.8 | 152 | 3.9 |
| Maprotiline | Level I | 80.4 | 2.5 | 85.7 | 3.5 | 82.8 | 2.6 | 83.0 | 3.2 |
| | Level II | 193 | 5.1 | 210 | 4.5 | 196 | 3.8 | 200 | 4.5 |
| Norclomipramine | Level I | 75.5 | 2.2 | 78.8 | 2.6 | 77.8 | 2.6 | 77.3 | 2.2 |
| | Level II | 179 | 4.3 | 189 | 4.7 | 182 | 3.2 | 183 | 2.7 |
| Norclozapine | Level I | 179 | 1.4 | 182 | 3.9 | 180 | 3.0 | 180 | 0.8 |
| | Level II | 419 | 3.9 | 431 | 3.4 | 417 | 3.6 | 422 | 1.7 |
| Nordoxepin | Level I | 47.4 | 3.5 | 50.1 | 2.4 | 49.7 | 2.0 | 49.1 | 3.0 |
| | Level II | 118 | 5.2 | 125 | 4.5 | 120 | 4.3 | 121 | 3.3 |
| Normaprotiline | Level I | 113 | 5.4 | 122 | 4.7 | 119 | 3.4 | 118 | 3.9 |
| | Level II | 284 | 4.6 | 316 | 5.2 | 285 | 5.0 | 295 | 6.2 |
| Nortrimipramine | Level I | 37.9 | 3.4 | 40.0 | 4.8 | 41.8 | 1.7 | 39.9 | 4.9 |
| | Level II | 93.7 | 6.2 | 100 | 5.7 | 101 | 4.1 | 98.2 | 4.0 |
| Nortriptyline | Level I | 66.7 | 3.1 | 69.9 | 4.0 | 70.3 | 3.2 | 69.0 | 2.9 |
| | Level II | 159 | 5.1 | 172 | 5.4 | 166 | 4.1 | 166 | 4.0 |
| Protriptyline | Level I | 58.2 | 3.2 | 61.8 | 3.9 | 60.8 | 3.7 | 60.3 | 3.1 |
| | Level II | 143 | 5.3 | 154 | 5.6 | 145 | 5.7 | 147 | 4.1 |
| Trimipramine | Level I | 103 | 1.7 | 109 | 3.8 | 113 | 3.7 | 108 | 4.8 |
| | Level II | 249 | 5.3 | 267 | 4.8 | 270 | 4.3 | 262 | 4.4 |

Table 7. LLOQs for all compounds

| Analyte | LLOQ (µg/L) |
|---|---|
| Amitriptyline | 3.26 |
| Clomipramine | 3.66 |
| Clozapine | 11.7 |
| Desipramine | 3.62 |
| Doxepin | 2.96 |
| Imipramine | 3.34 |
| Maprotiline | 4.84 |
| Norclomipramine | 4.22 |
| Norclozapine | 2.37 |
| Nordoxepin | 2.72 |
| Normaprotiline | 18.2 |
| Nortrimipramine | 16.9 |
| Nortriptyline | 3.64 |
| Protriptyline | 3.38 |
| Trimipramine | 1.37 |

## Conclusion

A robust, reproducible, and sensitive liquid chromatography-tandem mass spectrometry method for clinical research for quantification of fifteen tricyclic antidepressants in human plasma was developed and implemented. The analytical method was validated on an Vanquish Flex Binary UHPLC system coupled to a TSQ Quantis triple quadrupole mass spectrometer. Method performance was evaluated using the ClinMass LC-MS/MS calibrators, controls, and internal standards. The method described here offers quick and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

Find out more at **thermofisher.com/ClinicalResearchApps**


**TN66052-EN 0621S**

ThermoFisher SCIENTIFIC