

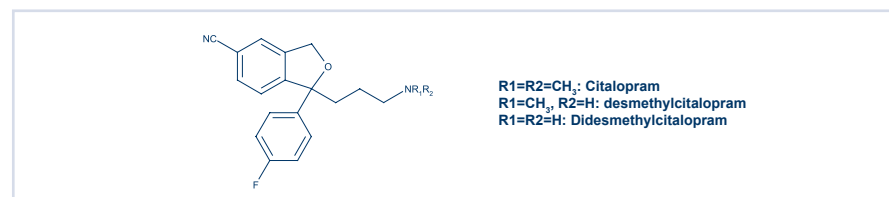
Determination of Citalopram and its Metabolites Desmethylcitalopram and Didesmethylcitalopram in Human Plasma by Liquid-liquid Extraction and LC/MS/MS

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Introduction:

This presentation provides details about the development and validation of an LC/MS/MS method for the determination of citalopram, an antidepressant, and its metabolites desmethylcitalopram and didesmethylcitalopram in K₂-EDTA human plasma.



Objective:

- To develop an efficient, accurate and rugged procedure for the determination of citalopram and its metabolites in K₂-EDTA human plasma by LC/MS/MS.
- To validate analysis of citalopram and its metabolites over the ranges 1.00 – 100 ng/mL and 0.250 – 25.0 ng/mL, respectively.
- To quantify citalopram and its metabolites in plasma samples collected from dosed human subjects.

Methodology:

Chemicals

Reference material and internal standards (citalopram-D₄ and desmethylcitalopram-D₄) were obtained from SynFine Research, Inc. All solvents were HPLC grade and additional reagents were ACS Reagent grade or better.

Sample Preparation

Individual stock solutions of citalopram, desmethylcitalopram and didesmethylcitalopram as the hydrobromides were prepared in acetonitrile/water (1:1,v/v), and these stocks were used to prepare a combined intermediate solution in acetonitrile/water at a known concentration of each analyte. Calibration standard spiking solutions covering the required ranges were then prepared in the same solvent. Stock solutions of citalopram-D₄ and desmethylcitalopram-D₄ were prepared similarly in acetonitrile/water (v/v), and a combined internal standard working solution in the same solvent was prepared from these stocks.

For validation, Quality Control samples were prepared in K₂-EDTA human plasma at three different concentration levels (QC Low = 3.00 ng/mL for citalopram, 0.750 ng/mL for the metabolites; QC Medium = 20.0 ng/mL for citalopram, 5.00 ng/mL for the metabolites; QC High = 80.0 ng/mL for citalopram, 20.0 ng/mL for the metabolites). These QC samples were stored in 0.200 mL aliquots at -20°C.

Extraction

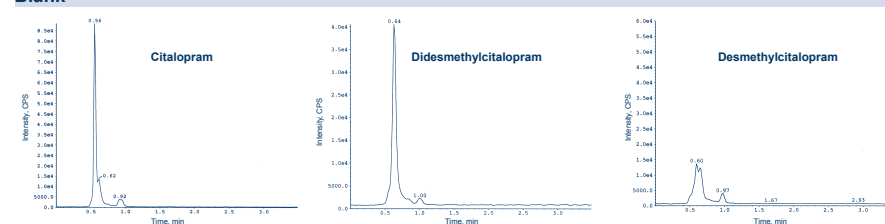
- Aliquot 0.200 mL K₂-EDTA human plasma.
- Spike the standards with 20.0 µL spiking solution.
- Add 20.0 µL of working internal standard solution to the standards, blanks + IS and QC's.
- Add 100 µL of methanol and 500 µL 1M sodium carbonate solution.
- Add 2 mL of ethyl acetate/hexanes 9:1, vortex vigorously and centrifugate at 3000 rpm for 10 min.
- Transfer the organic layer and evaporate with a gentle stream of nitrogen at 40°C for 10 min.
- Reconstitute in 200 µL of mobile phase A.
- Analyze by LC-MS-MS.

Sample Analysis by LC-MS-MS

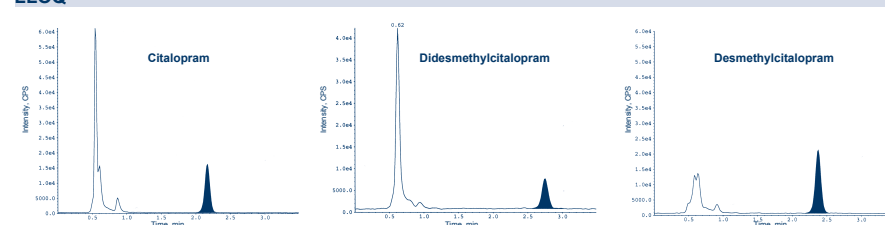
The HPLC system consisted of a Waters Inc. PVASil column (4x50 mm) and a gradient from 2 to 95% of mobile phase B at 0.8 mL/min. Mobile phase A was acetonitrile/water/formic acid/ammonium hydroxide (950:50:2:0.2,v/v) and mobile phase B was acetonitrile water (1:1, v/v). Mass spectrometry detection was carried out on a Sciex API 5000 equipped with a TurboIonSpray source. Mass spectra were acquired in positive ion mode with MRM monitoring of ion transitions 325→109 for citalopram, 311→109 for desmethylcitalopram, and 297→109 for didesmethylcitalopram.

Representative Chromatograms:

Blank



LLOQ



Results:

Standard Precision and Accuracy

Citalopram: Mean of Three Validation Runs

Amount Added, ng/mL	1.00	2.00	5.00	10.0	20.0	50.0	90.0	100
Mean Found, ng/mL	1.03	1.91	4.98	10.1	19.7	50.5	89.9	103
CV (%)	2.0	5.0	3.6	1.5	1.1	1.8	1.1	2.0
% Bias	3.0	-4.5	-0.4	1.0	-1.5	1.0	-0.1	3.0

Desmethylcitalopram: Mean of Three Validation Runs

Amount Added, ng/mL	0.250	0.500	1.25	2.50	5.00	12.5	22.5	25.0
Mean Found, ng/mL	0.250	0.501	1.24	2.56	4.94	12.6	22.2	25.2
CV (%)	0.4	0.8	2.1	1.3	1.0	1.7	0.9	2.4
% Bias	0.0	0.2	-0.8	2.4	-1.2	0.8	-1.3	0.8

Didesmethylcitalopram: Mean of Three Validation Runs

Amount Added, ng/mL	0.250	0.500	1.25	2.50	5.00	12.5	22.5	25.0
Mean Found, ng/mL	0.253	0.497	1.19	2.53	4.87	12.8	22.4	25.9
CV (%)	0.0	0.9	3.2	1.0	6.1	2.7	2.5	0.2
% Bias	1.2	-0.6	-4.8	1.2	-2.6	2.4	-0.4	3.6

LLOQ and QC Precision and Accuracy

Citalopram: Mean of Three Validation Runs (n=18)	Low	Med	High	
Amount Added, ng/mL	1.00	3.00	20.0	80.0
Mean Found, ng/mL	1.01	2.95	19.7	76.3
CV (%)	5.1	2.0	1.5	2.5
% Bias	1.0	-1.7	-1.5	-4.6

Desmethylcitalopram: Mean of Three Validation Runs (n=18)

Amount Added, ng/mL	0.250	0.750	5.00	20.0
Mean Found, ng/mL	0.250	0.751	5.11	19.6
CV (%)	3.2	1.0	1.4	1.8
% Bias	0.0	0.1	2.2	-2.0

Didesmethylcitalopram: Mean of Three Validation Runs (n=18)

Amount Added, ng/mL	0.250	0.750	5.00	20.0
Mean Found, ng/mL	0.244	0.780	5.30	21.1
CV (%)	3.6	2.7	1.9	2.5
% Bias	-2.4	4.0	6.0	5.5

Stability

Citalopram

Concentration, ng/mL	3.00	80.0
Mean % Change		
BTS, 24 hrs @ 22°C	1.6	-2.0
FTS, 5 cycles	1.4	-3.6
XTS, 71 hrs @ 22°C	6.5	-1.1
LTS, 75 days @ -20°C	1.9	4.0

Desmethylcitalopram

Concentration, ng/mL	0.750	20.0
Mean % Change		
BTS, 24 hrs @ 22°C	-0.3	-0.5
FTS, 5 cycles	1.1	-0.5
XTS, 71 hrs @ 22°C	1.3	-0.5
LTS, 75 days @ -20°C	0.2	1.8

Didesmethylcitalopram

Concentration, ng/mL	0.750	20.0
Mean % Change		
BTS, 24 hrs @ 22°C	5.3	3.0
FTS, 5 cycles	2.4	1.7
XTS, 71 hrs @ 22°C	5.9	2.8
LTS, 75 days @ -20°C	-2.4	5.7

No matrix interferences were observed for citalopram or the metabolites in six different lots of plasma. Acceptable intraday and interday precision (< 5.1% CV) and accuracy (< 7.0% bias) were found over the range of 1-100 ng/mL for citalopram and 0.25-25.0 ng/mL for the metabolites. The mean correlation of coefficients (1/x² weighting) were 0.9986 for citalopram, 0.9996 for desmethylcitalopram and 0.9982 for didesmethylcitalopram. The recoveries at three different levels were 82.4 to 86.4% for citalopram, 83.9 to 87.7% for desmethylcitalopram and 83.1 to 92.0% for didesmethylcitalopram. Stability was determined to be acceptable in plasma for 24 hours at room temperature, for 5 cycles of freeze/thaw, and for 71 hours in the extract form.

Conclusion:

An LC-MS-MS method for quantitation of citalopram and its metabolites in K₂-EDTA human plasma has been developed. This method was shown to be specific, accurate, and rugged, and has been validated for use in pharmacokinetic studies. The method has been used successfully to run clinical samples.

Note: After validation was finished, didesmethylcitalopram-D₆ became available. This internal standard was successfully used in a partial validation (data not shown) and for sample analysis.