Supporting Information

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Stability-indicating ultra-fast liquid chromatographic analysis of maprotiline in pharmaceutical formulations

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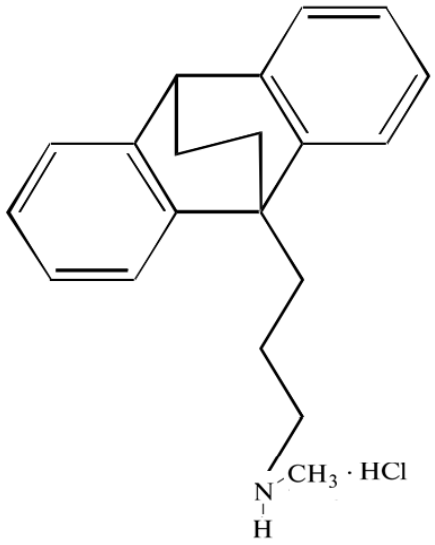


Figure S1 : Chemical structure of Maprotiline Hydrochloride

S1. Experimental

S.1.1. Chemicals and Reagents

Maprotiline hydrochloride was purchased from Sigma-Aldrich. Pharmaceutical formulations of MAP Ludiomil 25® mg was taken from local markets. All chemicals and reagents were of analytical-reagent grade. HPLC grade ultrapure water was prepared by an Elga Purelab Option Q (VWS Deutschland) water purification system.

S.1.2. Instrumentation and Chromatographic Conditions

The chromatographic separations were performed on a Shimadzu LC 20A UFLC (Shimadzu, Kyoto, Japan). A system was consisted of LC 20AB Binary pump, SIL 20AC Autosampler, CTO-10As column oven, and SPD M20A photodiode array (PDA) detector. Chromatographic data were collected and computed by LC Solution system software. Chromatographic separation was achieved on a Inertsustain C18 column (4.0 x 100 mm, 3 µm) (GL Sciences, Tokyo-JAPAN) under isocratic elution with acetonitrile and phosphate buffer buffer of pH:7 (75 : 25, v/v), at a flow rate of 0.4 mL per minute. The detection was carried out at 215 nm and the column temperature was set at 25 ºC.

The pH was measured by WTW pH 526 digital pH meter (Mettler Toledo, Germany).

S.1.3. Preparation of Stock Solution

A stock standard solution equivalent to 100 µg/mL of MAP was prepared by dissolving accurately weighed 10 mg of pure MAP in a 100 mL volumetric flask and diluted to mark with the asetonitrile:water (75:25) mixture and filtered through a 0.22 µm nylon membrane filter. The final concentrations of each drug were 0.1–1.5 µg/mL. The stock solutions were stored at 4 ºC and were stable for a month.

Phosphate buffer preparation: 72.75 mL of 0.2 M NaOH was taken in 1000 mL volumetric flask to it 125 mL of 0.2 M KH_2PO_4 was added and the volume was made up to 1000 mL with water.

S.1.4.Preparation of Sample Solution

10 mg tablets was accurately weighted and transferred to a 100 ml volumetric flask. A 50 mL of asetonitrile:water (75:25) mixture was added and and sonicated for 30 minutes. Then the volume was made up to the mark with same mixture and centrifuged for 10 minutes at 3000 rpm. The centrifuged solution was filtered with 0.45 µm syringe filter. Appropriate dilutions were made with asetonitrile:water (75:25) mixture to obtain final concentrations.

S.1.5. Stress Studies

Forced degradation studies were carried out to test the stability-indicating properties and specificity of the proposed method to determine MAP in pharmaceutical formulation and bulk. Forced degradation procedures were conducted by exposing the drug substance to acidic, basic, neutral, oxidative, photolytic, and thermal stress conditions.

S.1.5.1. Acid Hydrolysis

To conduct the acid decomposition studies, the sample of MAP bulk was dissolved in 0.1 M HCl to attain a concentration of 1 µg/mL. Then, the solution was heated to 80°C and this temperature was maintained

for 4 hours. Before performing the UFLC analysis, the samples were diluted appropriately and neutralized with 0.1 N NaOH.

S.1.5.2. Base Hydrolysis

The samples were exposed to alkaline conditions by mixing them with 0.1 M NaOH to attain a concentration of 1 µg/mL. Following this step, the solution was heated to reach 80°C and this temperature was maintained for 4 hours. Before the UFLC analysis, the elicited samples were diluted appropriately and neutralized with 0.1 N HCl.

S.1.5.3. Oxidation

To study the oxidative degradation, the drug substance was treated with 3% hydrogen peroxide solution at room temperature for 4 hours.

S.1.5.4. Thermal Degradation Studies

To carry out the thermal stress study, the drug product was kept in a temperature-controlled oven at 105°C for 1 hour.

S.1.5.6. Photolytic Degradation Studies

To test for the photolytic degradation, the drug substance was exposed to daylight for 24 hours.

S.2. Method Validation

S. 2.1. Linearity and Range

The solution of MAP was prepared at five different concentrations from 0.1 to 1.5 µg/mL of analyte concentration. The regression line was plotted with area versus concentration using the method of least squares analysis. The values of the slope and Y-intercept of the plot were calculated.

S.2.2. Accuracy

The accuracy of method was determined by calculating recoveries by spiking method. Known amount of standard solutions of 0.1, 0.5, 1.0 µg/mL were spiked into known amount sample solutions (1µg/mL). The amount was estimated by the regression equation of the calibration curve.

S.2.3. Precision

The precision of the method was determined in terms of intra-day precision and inter-day precision. The inter- and intra-day precisions were examined by analysis of MAP with the three concentrations (n=5) for five consecutive days. The precision was given as the relative standard deviation (RSD%).

S. 2.4. Sensitivity

Sensitivity of the method was proved by establishing the limits of detection (LOD) and quantitation (LOQ) for MAP. The limit of detection (LOD) and the limit of quantitation (LOQ) were determined based on the 3.3 and 10 times the standard deviation of the response, respectively, divided by the slope of the calibration curve.

S. 2.5.Robustness

The robustness of the method was studied by deliberately changing method parameters like flow rate of the mobile phase, detection wavelength, organic phase composition and column temperature (25 ± 5°C). Solution stability of the drug in the mobile phase was determined by keeping the drug solution at ambient conditions for 24h.

Table S1: Results of recoveries data of MAP

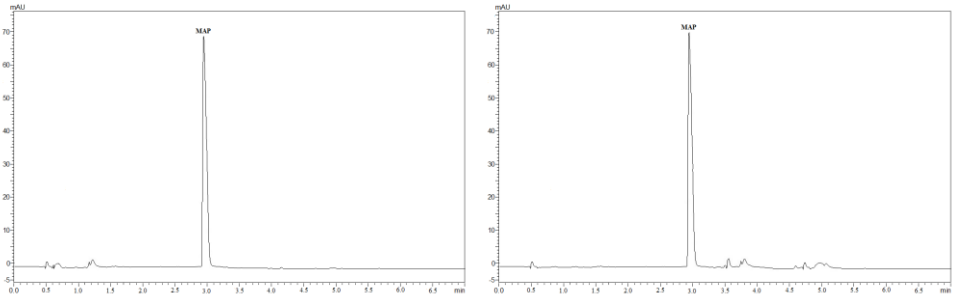
MAP	Amount taken[a](µg/mL)	Amount Added (µg/mL)	Total amount found [b] (µg/mL) (Mean± S.D.[c])	Recovery	RSD
	1.0	0.1	1.11±0.009	100.91	0.81
		0.5	1.49±0.012	99.33	0.80
		1.5	2.53±0.024	101.20	0.30

[a] Ludiomil *Tablet*®(25 mg)
[b]Five independent analyses.
[c]Standard deviation

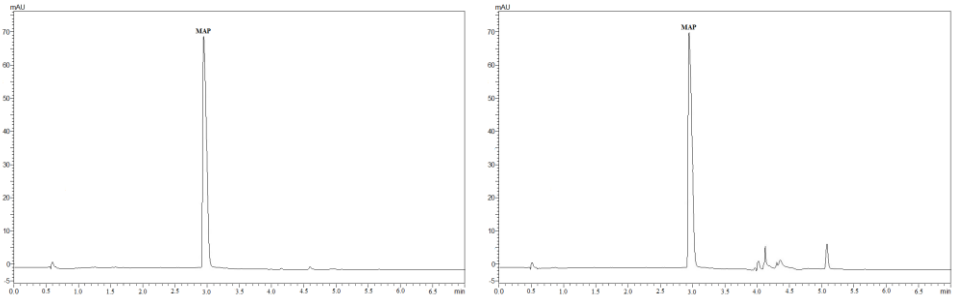
Table S2: Forced degredation results

Stress Condition	Recovery (%)	Purity index	Purity Threshold
Acid hydrolysis	74.35	0.999794	0.999891
Base hydrolysis	87.76	0.999854	0.999899
Oxidation	82.84	0.999867	0.999841
Thermal degradation	98.69	0.999787	0.999889
Day light degradation	99.71	0.999886	0.9998692

(A) (B)



(C) (D)



(E) (F)

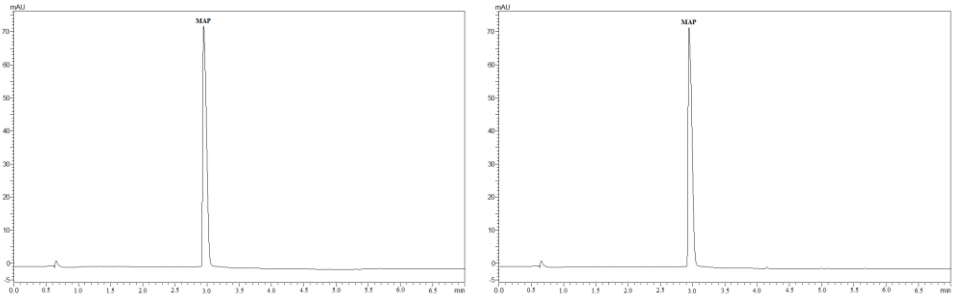


Figure S2 : Chromatograms of MAP 1.0 µg/mL (A) acid-degraded drug, (B) base-degraded drug, (C) water hydrolysis-degraded drug, (D) oxidation-degraded drug, (E) thermal-degraded drug and (F) daylight-degraded drug