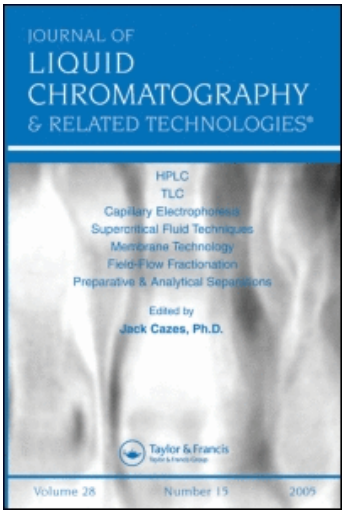

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Jason T. Johnson[a]; Samuel W. Oldham[a]; Ronald J. Lantz[a]; Allyn F. DeLong[a]
[a] Lilly Research Laboratories Lilly Laboratory for Clinical Research Eli Lilly and Co., Indianapolis, IN, USA

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF DULOXETINE AND DESMETHYL DULOXETINE IN HUMAN PLASMA

Jason T. Johnson, Samuel W. Oldham,
Ronald J. Lantz, Allyn F. DeLong

Lilly Research Laboratories
Lilly Laboratory for Clinical Research
Eli Lilly and Co.
Indianapolis, IN 46202, USA.

ABSTRACT

A high performance liquid chromatographic (HPLC) method was developed and validated for the analysis of duloxetine and desmethyl duloxetine in human plasma. Plasma was adjusted to pH 10 with 1.0 M sodium carbonate and extracted with hexane which contained 2% isopropyl alcohol. The concentrated extract was derivatized with dansyl chloride (500 µg/mL). A Phenomenex Primesphere 5 C_{18} HC column provided chromatographic separation of the analytes followed by fluorescence detection with excitation and emission wavelengths at 285 nm and 525 nm respectively. The linear dynamic range for both analytes was 2 ng/mL to 64 ng/mL with precision and accuracy of <10%.

INTRODUCTION

Animal models of pharmacologic activity demonstrated that duloxetine hydrochloride is a potent and selective mixed serotonin (5-HT) / norepinephrine (NE) reuptake inhibitor.[1-3] After single and multiple once a day dose administration of 5 mg to 40 mg in volunteers, duloxetine blocked the uptake of serotonin in human platelets and was well tolerated by the subjects. Duloxetine hydrochloride is currently being evaluated in depression and other disease states for which alteration of serotonergic and noradrenergic neurotransmission may produce beneficial therapeutic effects.

A high performance liquid chromatographic (HPLC) assay was developed to monitor duloxetine and desmethyl duloxetine during clinical trials and to determine the pharmacokinetics of duloxetine and metabolite.

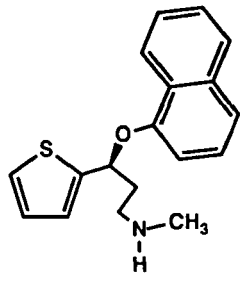
EXPERIMENTAL

Chemicals and Reagents

Desmethyl duloxetine, 210980 (I), duloxetine hydrochloride, LY248686 (II), and internal standard, 113821 (III), were synthesized at Lilly Research Laboratories (Eli Lilly and Co., Indianapolis, IN, USA (Fig. 1, structures, base forms). HPLC quality water was prepared from a Millipore Milli-Q System (Marlborough, MA, USA). Acetone, acetonitrile, and methanol were HPLC grade and purchased from Mallinckrodt, Paris, KY, USA. Hexane and isopropyl alcohol were purchased from Baxter Scientific, USA. Ammonium acetate was purchased from EM Science, USA. Dansyl chloride was purchased from Sigma, USA. Sodium carbonate anhydrous was purchased from Mallinckrodt. Drug free (blank) heparinized human plasma was purchased from Biological Specialty Corporation, Colmar, PA, USA.

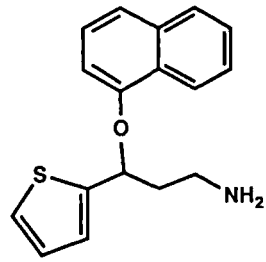
High Performance Liquid Chromatography

The HPLC system consisted of a Waters 715 Ultra WISP (Waters Associates, Millford, MA, USA), Beckman System Gold 126 binary pumping system (Beckman Instruments, Inc., San Ramon, CA, USA), Perkin Elmer LS40 Fluorescence detector (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England) and Perkin Elmer Access*Chrom data reduction system. The guard column / analytical column combination was a Zorbax ODS, 4 mm x 1.25 cm, 5 μm (MAC - MOD, Chadds Ford, PA, USA) / Phenomenex



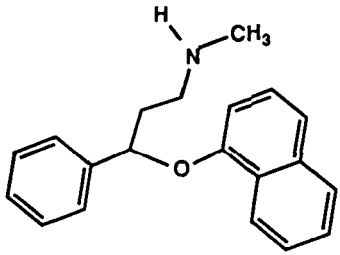
DULOXETINE (LY248686) FREE BASE

(II)



DES-METHYL METABOLITE (210980)

(I)



INTERNAL STANDARD (113821)

(III)

Figure 1. Structures (base form) of desmethyl duloxetine (I), duloxetine (II), and internal standard (III).

Primesphere 5 C_{18} HC, 25 cm x 4.6 mm, 5 µm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 21% 0.05 M ammonium acetate/79% acetonitrile which was filtered and degassed. The flow-rate was 2.0 mL/min and the column temperature was ambient. The chromatographic run required 20 minutes for completion. The analytes were detected and quantified by fluorescence at excitation and emission settings of 285 nm and 525 nm, respectively.

Preparation of Standard Solutions

Silylated glassware and selected plasticware were used to prepare the samples and standards to minimize the nonspecific adsorption of the analytes to glassware. Stock solutions of I, II, and III were prepared in methanol at free base concentrations of 100 μg/mL. The plasma calibrators were prepared by spiking an appropriate amount of plasma with the standard stock to yield a 64 ng/mL sample. This sample was diluted with blank plasma to obtain calibrators of 32, 16, 8, 4, and 2 ng/mL.

Quality control samples at concentrations of 50, 25, and 5 ng/mL were prepared from a separate weighing of compound. A standard curve was prepared for each validation run in which 5 samples of each quality control concentration were analyzed. The quality control samples were stored at -20°C.

Sample Preparation Procedures

One mL aliquots of quality control samples and freshly prepared calibrators were placed in individual silylated 16 mm x 125 mm screw cap tubes with Teflon caps (Kimble Series 73750). After the addition of 1 mL of 1.0 M sodium carbonate buffer, pH 10, 200 μl of a 0.1 μg/mL internal standard solution, and 6 mL of a 98% hexane / 2% isopropyl alcohol solution, the tubes were tightly sealed with the screw cap. The samples were placed on a Labquake shaker (Labindustries Inc., Berkeley, CA, USA.) for 45 minutes. The samples were centrifuged at approximately 825 x g for 10 minutes and then placed in a methanol / dry ice bath to freeze the aqueous component.

The upper organic layer was decanted from the lower frozen aqueous layer and placed in 13 mm x 100 mm silylated culture tubes (Kimble Series 73500). The tubes containing the organic phase were placed in a Multivap (Organomation Associates, Berlin, MA, USA) sample concentrator at about 42°C for approximately 30 minutes or until dry.

The dried residue was then derivatized by adding 2 mL of acetone, 150 μl of 0.1 M sodium carbonate, pH 10, and 75 μl of 500 μg/mL dansyl chloride at 55°C for 30 minutes. The samples were reduced to dryness under nitrogen at 42°C and reconstituted with 200 μl of mobile phase. The samples were filtered using 10 μm filter tips (Zymark Corp., USA.) and 100 μl was injected onto the column. A typical chromatographic separation of desmethyl duloxetine, duloxetine, and internal standard from human plasma and a plasma standard can be seen in Fig. 2.

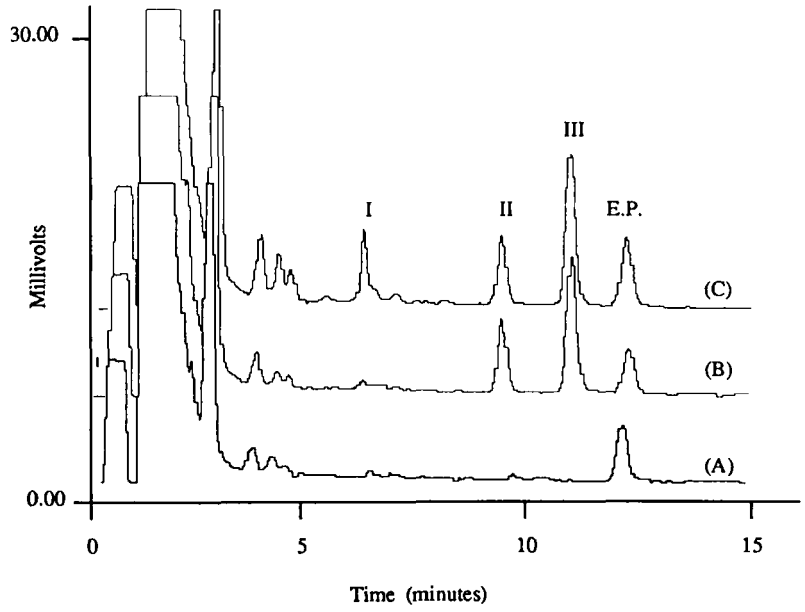


Figure 2. Representative chromatograms of blank human plasma (A), plasma sample with <LOQ (I) and 9.2 ng/mL (II) (B), and a plasma standard with 8 ng/mL (I) and 8 ng/mL (II) (C). Peaks: I = desmethyl duloxetine, II = duloxetine, III = internal standard. <LOQ represents below the limit of quantification which is 5 ng/mL. E.P. represents an endogenous peak.

Data Reduction / Data Acceptability Criteria

Data reduction/calculation was accomplished with an on line computer system (Perkin Elmer Access*Chrom). Calculation drug concentrations in unknown samples was based on a weighted ($1/x^2$) least squares regression of plasma calibrator concentrations against the peak height ratios. The peak height ratios were obtained by dividing the peak heights of I and II by the peak height of the internal standard.

Each five (5) point calibration curve must have a coefficient of determination of at least 0.97. Both the relative error and the relative standard deviation of the control samples must be <15% of the theoretical value except for those at the limit of quantification which must be < 20%.

Determination of Standard Curve Characteristics, Precision and Accuracy

The regression coefficients for duloxetine and desmethyl duloxetine were greater than 0.99. The back calculated results for the standard curve demonstrated the precision and accuracy of the standard curves (Table 1). The precision and accuracy of the HPLC assay were evaluated by performing statistical analysis of 5 replicates of each quality control concentration during 3 days (Table 2). The statistical analysis of the precision and accuracy data demonstrate that the assay is suitable for quantifying the two analytes during clinical trials and/or pharmacokinetic studies to a limit of quantification of 5 ng/mL.

Determination of Drug/Metabolite Stability in Plasma

The stability of duloxetine and desmethyl duloxetine was determined at room temperature, -20°C, and -70°C. Also, the effects of freeze-thaw cycles were determined. At a minimum, replicate samples of known concentrations of the analytes were analyzed. Both analytes were stable at concentration ranges of 5 to 50 ng/mL at -20°C and -70°C for at least 2 months. The compounds were unaffected after three freeze-thaw cycles at -20°C. A sample with an approximate concentration of 30 ng/mL of each compound was stable at room temperature for at least 24 hours.

RESULTS AND DISCUSSION

Chromatography

The majority of the drug related analytes found in human plasma after administration of duloxetine hydrochloride was the parent drug. The desmethyl metabolite was seen in trace amounts. The retention times of the desmethyl metabolite, parent drug and internal standard were approximately 6.1, 9.3, and 10.9 minutes, respectively (Figure 2).

Assay Specificity

The specificity of the assay was determined by analyzing several lots of blank plasma for interferences. It was found that all plasma screened contained an endogenous peak with an approximate retention time of 12.2 minutes. This

Table 1

Standard Curve Characteristics

DULOXETINE					DESMETHYL DULOXETINE				
Target Value ng/mL	Mean ng/mL n = 5	S.D.	RE (%)	RSD (%)	Target Value ng/mL	Mean ng/mL n = 5	S.D.	RE (%)	RSD (%)
2	2.04	0.06	2.17	2.95	2	2.13	0.15	6.5	6.82
4	3.98	0.09	-0.42	2.14	4	4.05	0.07	1.17	1.79
8	7.98	0.06	-0.025	0.78	8	7.81	0.15	-2.38	1.91
16	15.78	0.17	-1.4	1.05	16	14.95	1.15	-6.65	7.68
32	31.68	0.34	-1.01	1.06	32	31.8	0.3	-0.62	0.95
64	64.54	0.32	0.84	0.5	64	65.28	1.24	2.01	1.9

LINEAR REGRESSION ANALYSIS[1]

Standard Curve Characteristics

Day	K0	K1	R2
1	-0.0116	0.09525	0.09996
2	-0.0148	0.057	0.9998
3	-0.0005	0.0589	0.9999

LINEAR REGRESSION ANALYSIS[1]

Standard Curve Characteristics

Day	K0	K1	R2
1	0.0135	0.06560	0.9944
2	-0.0178	0.0686	0.9993
3	-0.0136	0.0712	0.9999

[1] K0 = y-intercept of standard curve
K1 = slope of curve
R2 = caofficient of determination
RE = Relative Error
RSD = Relative Standard Deviation

endogenous peak did not interfere with the internal standard peak. There were no interferences in the resolution of the analytes in this chromatographic system.

Overall Analyte Recovery and Assay Linearity

The extraction recovery for duloxetine, desmethyl duloxetine, and internal standard was 27%, 58%, and 25%, respectively. The recovery was determined by measuring the peak height of extracted plasma samples vs. the peak height of neat standards in methanol.

As observed in blank plasma (Figure 2), hexane extraction provided minimal background from plasma components while providing consistent recovery of analytes. The standard curve characteristics and back calculated values of the standard curve samples are shown in Table 1. The assay was linear from 2 ng/mL to 64 ng/mL for both duloxetine and desmethyl duloxetine.

Precision, Accuracy, and Limit of Quantification

The determination of I and II was evaluated for precision and accuracy by replicate analysis of the quality control pools at three different concentrations within the standard curve range (Table 2). Both within-day and between-day precision and accuracy was found to be less than 10%. In addition, the limit of quantification (LOQ) was found to be 5 ng/mL.

Use of the Method During Patient Clinical Trials

The method was used to measure the concentration of the analytes in human plasma samples from clinical studies. Figure 3 displays plasma concentrations of duloxetine from a pilot bioequivalency study. The plasma concentrations of desmethyl duloxetine were mainly below the limit of assay quantification.

Conclusion

The three day validation data for this method shows that the relative error and relative standard deviation for both duloxetine and desmethyl duloxetine were < 10%. However, it has been found with application of this method that

Table 2

Precision and Accuracy for Duloxetine and Desmethyl Duloxetine

		DULOXETINE			DESMETHYL DULOXETINE		
		Target Concentration (ng/mL)			Target Concentration (ng/mL)		
		50.00	25.00	5.00	50.00	25.00	5.00
Day 1[1]	Mean	51.31	25.60	5.08	50.56	26.15	4.68
	S.D.	0.95	0.70	0.21	3.74	1.84	0.16
	RE (%)	2.62	2.40	1.52	1.12	4.58	-6.40
	RSD (%)	1.86	2.75	4.08	7.39	7.03	3.49
Day 2[1]	Mean	46.89	22.85	4.75	49.60	24.39	4.93
	S.D.	0.20	0.07	0.22	1.00	0.68	0.13
	RE (%)	-6.22	-8.60	-4.96	-0.79	-2.45	-1.32
	RSD (%)	0.43	0.29	4.72	2.02	2.78	2.62
Day 3[1]	Mean	45.68	21.97	4.51	49.75	24.21	5.10
	S.D.	0.56	0.14	0.12	2.18	0.78	0.19
	RE (%)	-8.63	-12.14	-9.80	-0.50	-3.14	2.04
	RSD (%)	1.22	0.64	2.60	4.38	3.23	3.78
Overall[2]	Mean	47.96	23.47	4.78	49.97	24.92	4.91
	S.D.	2.57	1.65	0.30	2.41	1.44	0.24
	RE (%)	-4.08	-6.11	-4.41	-0.06	-0.34	-1.89
	RSD (%)	5.37	7.02	6.22	4.83	5.80	4.79

(1) n = 5 determinations
(2) n = 15 determinations
S.D. = standard deviation
Accuracy expressed as %RE = relative error (%R.E. = ((mean/theoretical) -1)*100)
Precision expressed as %RSD = relative stadard deviation (%R.S.D. = (standard deviation/mean)*100)

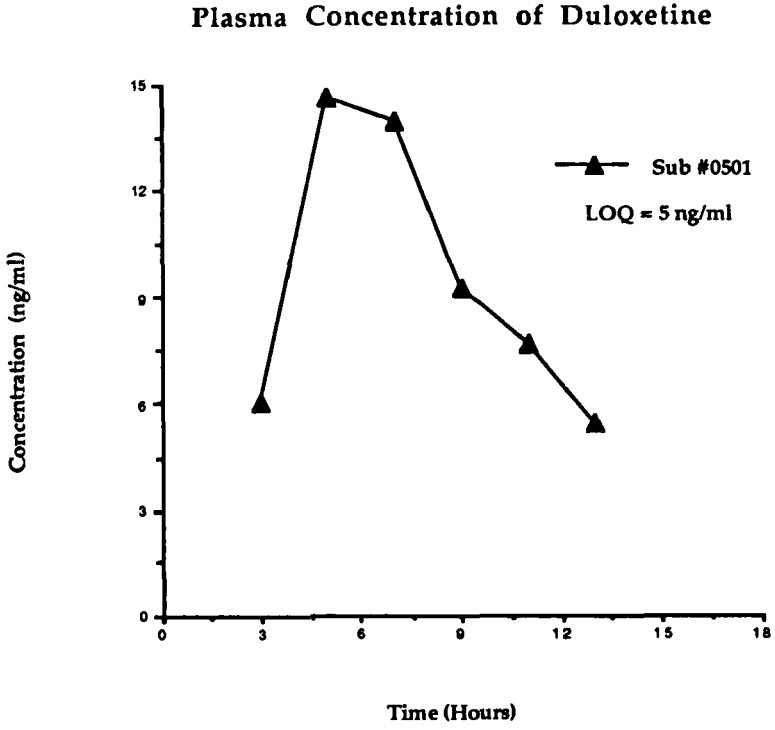


Figure 3. Plasma duloxetine in human subject #0501. Dose = 20 mg enteric coated tablet, fasted, morning.

the desmethyl duloxetine precision and accuracy data is sometimes > than 10%. The reason for the difference in the precision /accuracy of desmethyl duloxetine compared to duloxetine is unknown at this time.

This method has been used by our laboratory for bioavailability/bioequivalence studies and to monitor duloxetine plasma concentrations during clinical studies.

REFERENCES

1. D. R. Wong, F. P. Bymaster, D. A. Mayle, L. R. Reid, J. H. Krushinski, D. W. Robertson, LY248686, "A New Inhibitor of Serotonin and Norepinephrine Uptake," Neuropsychopharmacology **8**, 23, 1993.

2. R. W. Fuller, S. K. Hemrick-Luecke, H. D. Snoddy, "Effects of Duloxetine, an Antidepressant Drug Candidate, on Concentrations of Monoamines and their Metabolites in Rats and Mice, J. Pharmacol. Exp. Ther., **269**, 132, 1994.

3. T. Kihara, M. Ikeda, "Effects of Duloxetine, a New Serotonin and Norepinephrine Uptake Inhibitor, on Extracellular Monoamine Levels in Rat Frontal Cortex, J. Pharmacol. Exp. Ther., **272**, 177, 1995.

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