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Determination of Clozapine and its Metabolite, Norclozapine in Various Biological Matrices Using High-Performance Liquid Chromatography

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Purpose. To develop and validate an HPLC method for the quantitation of clozapine and its metabolite, norclozapine in human plasma, rat plasma, and the various human plasma lipoprotein fractions. Methods. Using liquid-liquid extraction, clozapine, and norclozapine are extracted from the biological matrix with MTBE. After concentration, the residue was reconstituted in 1mM HCl and injected on to a C6 Phenyl column (3µm, 2.0×150 mm). Mobile phase was 10mM Ammonium Acetate, pH 5—Acetonitrile—Methanol (5:3:2, v/v/v). Loxapine served as the internal standard. Absorbance was measured at 254 nm. Results. Quantitation limits for clozapine and norclozapine was 100 ng/mL and 50 ng/mL, respectively. Recovery for both clozapine and norclozapine was near 100%. Percent accuracy for intraday variability in human plasma, rat plasma, and human TRL, HDL, LDL, and LPDP lipoprotein fraction was between 92 to 113% for both analytes. Intraday precision for the same matrices was less than 9% CV for both analytes. Percent accuracy and precision for interday variability in human plasma was 97 to 103% and less than 10% CV, respectively. Samples were stabile in the autosampler for 80 h at 10°C and on the benchtop for 2 h. It should be noted, Clozapine-N-oxide, which is a known metabolite of Clozapine, was not determined since it is not clinically active. Conclusions. This method is a simple, fast and robust HPLC assay for the determination of clozapine and norclozapine in various matrices and lipoprotein fractions.

Keywords clozapine; norclozapine; HPLC; rat plasma; human plasma; lipoprotein fractions

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INTRODUCTION

Clozapine is an atypical, second generation, anti-psychotic commonly used in the treatment of schizophrenia. Originally removed from the market due to incidents of agranularcytosis, clozapine was reintroduced as it was found to be effective against refractory schizophrenia and employed regular safety monitoring to help reduce the incidents of toxicity. When compared to other atypical antipsychotics, the full mechanism of action of clozapine has not been fully uncovered, however it is known to have affinity to the 5-HT_{2A}, D_2, and D_4 receptors (among others) acting in an antagonistic fashion (Ananth et al., 2004; Byerly & DeVane, 1996; Horacek et al., 2006; Khan et al., 2005).

Clozapine has been shown to be metabolized by CYP1A2 and CYP3A4 to form 2 major metabolites, norclozapine (*N*-desmethylclozapine) and clozapine N-oxide, respectively (Khan & Preskorn, 2005). Of interest to our research is the norclozapine metabolite because of its possible activity at the 5-HT_{1C}, 5-HT_2, and D_2 receptors (Kuppamaki et al., 1993) as

this may have a role in the efficacy of clozapine itself. It should be noted, clozapine-N-oxide, which is a known metabolite of clozapine, was not determined since it is not clinically active (Kuppamaki et al., 1993).

It has been shown in previous work in our lab that clozapine, a lipophilic compound, has altered in vitro distribution in lipoprotein fractions in plasma that contains elevated total cholesterol and triglyceride levels when compared to plasma of normal levels of total cholesterol and triglycerides (Procyshyn et al., 2001). This altered distribution in lipoproteins may lead to a change in the bioavailability of clozapine. In addition, because of altered lipoprotein distribution in varying plasma lipid profiles, we have also investigated the roll other lipophillic compounds may have on the distribution of clozapine, specifically how it may displace clozapine and alter its bioavailability (Honer et al., 2006; Procyshyn et al., 2005). In vitro studies of this type typically use a radiolabel which allows for good sensitivity and few concerns about recovery, but there are several drawbacks, primarily cost and safety when in vivo use is considered. Although many assays have been developed for the quantitation of clozapine and norclozapine in plasma (Ma & Lau, 1998; Mosier et al., 2003), none, to the knowledge of the authors, exist for the quantitation in plasma lipoprotein fractions in addition to multi-species plasma matrices. For this reason, our objective was to develop a simple, accurate and reproducible assay utilizing high performance liquid chromatography (HPLC) with ultraviolet (UV) detection for the quantitation of clozapine and its metabolite norclozapine in various biological matrices.

METHODS AND MATERIALS

Chemicals, Reagents, and Matrices

Blank, pooled human and rat plasma was purchased from Bioreclamation (Hicksville, NY). Triglyceride rich (TRL), low density lipoprotein, (LDL), high density lipoprotein (HDL), and lipoprotein deficient plasma (LPDP) lipoprotein fractions were isolated from blank, pooled human plasma as described elsewhere using density gradient ultracentrifugation (Procyshyn et al., 2001; Wasan et al., 1999). Clozapine and Norclozapine were purchased from American Radiolabeled Chemicals (St. Louis, MO). Loxapine and Methyl-Tert-Butyl Ether (MTBE) were purchased from Sigma (Oakville, ON). Na_2CO_3, 1 M Hydrochloric Acid (HCl), Glacial Acetic Acid, Acetonitrile (ACN), Methanol (MeOH) and Ammonium Acetate were all purchased from Fisher (Ottawa, ON). MTBE, ACN, MeOH were HPLC grade solvents. Distilled water was produced in house. Separate 1 mg/mL stock solutions of clozapine and norclozapine were prepared in MeOH for calibration standards and quality control samples. 1 mg/mL stock solution of loxapine internal standard (IS) was prepared in distilled water. Appropriate dilutions of clozapine and norclozapine stocks were made in MeOH for the preparation of combined clozapine-norclozapine working solutions for calibration standards and quality control samples.

HPLC

Validation was done on two HPLC instruments. One instrument contained a Waters 717 plus autosampler, Waters 600E controller, a Waters 486 Dual wavelength detector. Data was collected and analyzed with Shimadzu Class VP software. The second instrument was a Waters Alliance 2695 Separation unit with a 2996 PDA detector. Waters Empower 2 software was used to collect and analyze data from this instrument. Samples were kept at 10ºC in the autosampler. Separations were done with a Phenomenex Gemini C6-Phenyl, 3 µm, 2.0×150 mm column with matching Security Guard cartridge at 30°C. Mobile phase consisted of 10mM Ammonium Acetate (adjusted to pH 5 with Glacial Acetic Acid)-ACN-MeOH (5:3:2, v/v/v). Flow rate was set to 200 µL/min with a 10 µL injection volume. Run time was 16 min and absorbance was measured at 254 nm.

Design

For each extraction of the validation, a blank, blank with internal standard and 7 calibration standards (100—1000 ng/mL clozapine, 50—1000 ng/mL norclozapine) were extracted in the appropriate biological matrix. This range covered most of reported clozapine concentrations from clinically relevant doses in various studies (Khan & preskorn, 2005). Quality control samples were extracted in the same matrix as the calibration curve to determine the accuracy and precision of the assay. Quality control (QC) samples consisted of concentrations across the calibration range. For interday analysis, quality control samples were extracted and compared between days. For intraday analysis, quality control samples were extracted and compared to each other. Autosampler stability was determined by storing extracted quality control samples in the autosampler for 80 h at 10ºC before being analyzed. Extraction efficiency was determined by comparing extracted quality control samples with non-extracted quality control samples. Stability on the bench top was determined by preparing 3 of each quality control sample and storing the samples for 2 h on the bench top before extraction.

Extraction

Adapted from Mosier et al. (2003), 300 µL of blank matrix was aliquoted to labeled 12×75 mm culture tubes. To the calibration standards, 10 µL of diluted clozapine/norclozapine calibration working solution was added. To the quality control samples, 10 µL of diluted clozapine/norclozapine QC working solution was added. 10 µL of IS working solution (48 µg/mL) was added except to the blank and 100 µL of saturated Na_2CO_3 was added to all samples and vortexed to mix. 3 mL of MTBE was added, tubes were capped and vortexed on high for 5 min (VWR standard multi-tube vortexer). Tubes were centrifuged for 2 min at 3000rpm (Eppendorf 5810 R) and the organic

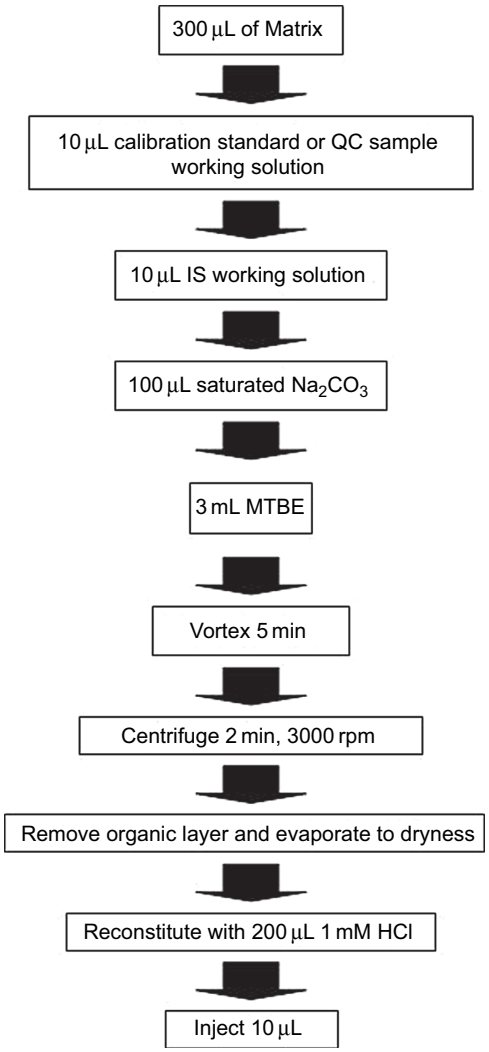

FIGURE 1. Extraction procedure flowchart.

layer was transferred to a correspondingly labeled 16×100 mm culture tube. Samples were evaporated to dryness with nitrogen in 50ºC water bath (Zymark Turbovap LV evaporator). Residue was reconstituted with 1mM HCl and transferred to autosampler vials (Figure 1).

Data Analysis

Chromatographic peaks were integrated and peak areas determined by the data collection software. Peak area ratios were calculated (Peak Area of analyte/Peak Area of IS) and calibration standards were plotted using weighted least square linear regression with $1/y^2$ weighting, as shown here (Almeida et al., 2002), to generate a calibration line. Peak area ratios of the calibration standards and quality control samples were compared against the calibration line and a concentration was determined. Percent accuracy was determined by comparing the nominal concentration with the calculated concentration ($C_{Calculated}/C_{Nominal}\times100$) of the quality control samples. Precision was determined by calculating percent Coefficient of Variation (%CV) of the quality control samples (standard deviation/mean $C_{Calculated}\times100$).

RESULTS

Chromatography of both extracted blank human and rat plasma were free from interfering peaks at the elution times of the analytes (Figures 2A and C). Norclozapine (~4 min), clozapine (~6 min) and the internal standard, loxapine (~9 min) are all well resolved from each other (Figures 2B and D). HDL lipoprotein fraction was also free from interferences at the elution times of the analytes and the approximate retention times of norclozapine, clozapine and loxapine were the same as above (Figures 2E and F). All remaining lipoprotein fractions had chromatography similar to that of the HDL lipoprotein fraction (data not shown).

All calibration curves contained 7 calibration points and were extracted from matrix as described above. Peak area ratios of norclozapine and clozapine to loxapine were calculated and the responses were plotted and a calibration line was determined as described above. All standard curves were linear ($R^2 > 0.98$) within the calibration range for both analytes. Calibration point responses were back calculated to the calibration line to determine how close the concentration was to its nominal value. All calibration points were ± 20% of nominal concentration (data not shown).

Interday reproducibility in human plasma was determined over 6 days. On each day, a calibration curve and 3 of each QC sample was extracted from human plasma. The calculated concentrations of the QC samples were averaged and compared to those on the other days. Norclozapine had an interday accuracy of 97 to 104% and precision of 4 to 10%. Clozapine had an interday accuracy of 98 to 103% and precision of 6 to 9% (Table 1). Intraday reproducibility in human plasma was determined in a single extraction of a calibration curve and 6 of each QC sample. The calculated concentration of each QC was compared to each other. Norclozapine had an intraday accuracy of 93 to 113% and precision of 2 to 7%. Clozapine had an intraday accuracy of 106 to 113% and precision of 2 to 8% (Table 2). Extraction efficiency in human plasma was determined by extracting a calibration curve, 6 of each LQC, MQC, and HQC and 18 blank plasma samples. The calibration curve and QC samples were extracted as described above. The 18 blank plasma samples were extracted as described above but the QC sample working solutions and the IS working solution were added after reconstitution with 1 mM HCl (6 of each LQC, MQC, and HQC). All extracted and non-extracted QC samples were compared to the calibration curve and concentrations determined. Extracted QC sample concentrations were compared to non-extracted concentrations and % extraction efficiency was calculated. Clozapine extraction efficiency was 101 to 107% and norclozapine extraction efficiency was 89 to 96%

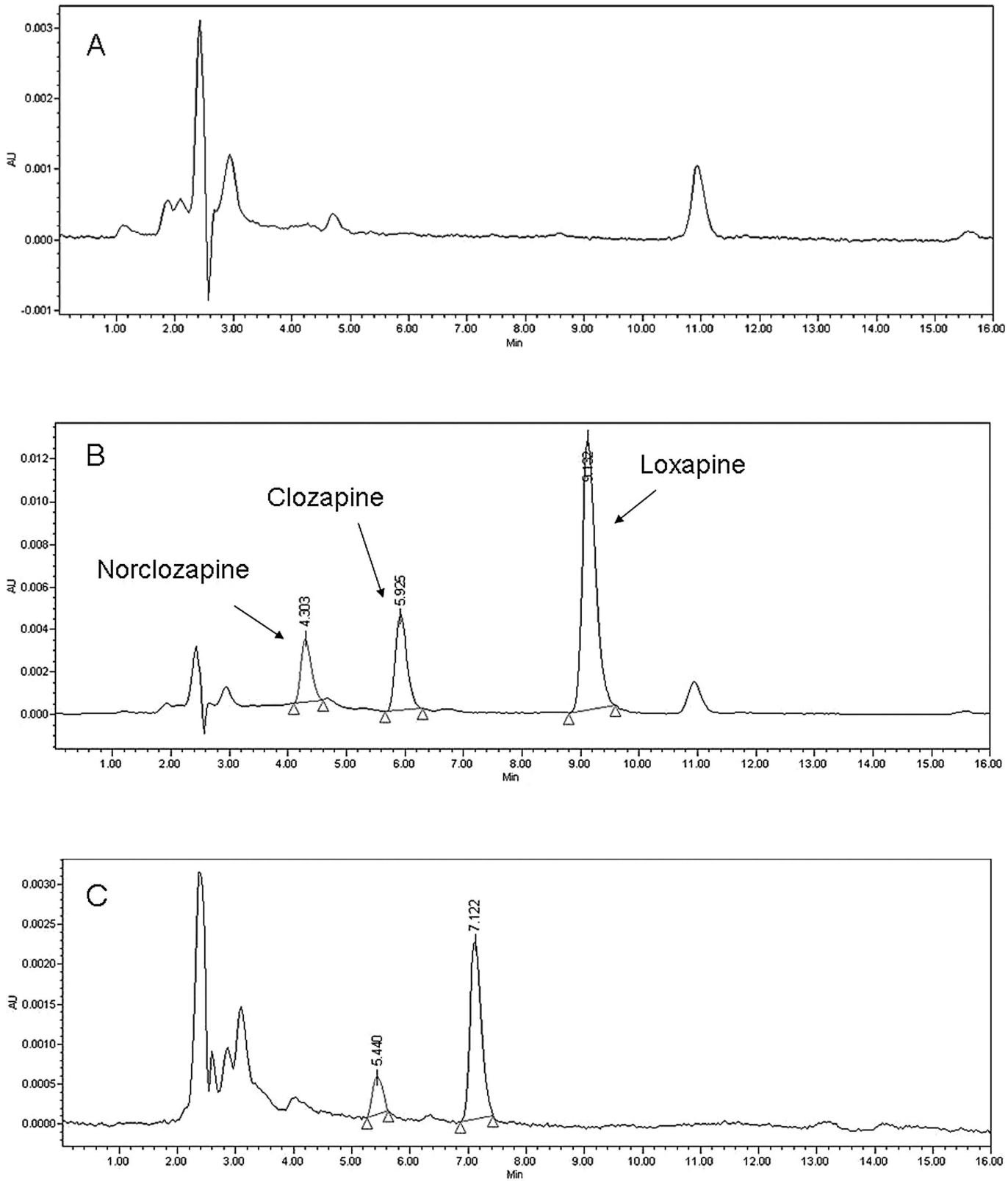

FIGURE 2. Representative chromatograms of Clozapine and norclozapine extracted from various biological matrices. (A) Blank human plasma. (B) Human plasma LQC. (C) Blank rat plasma. (D) Rat plasma LQC. (E) Blank human HDL fraction. (F) Human HDL fraction LQC.

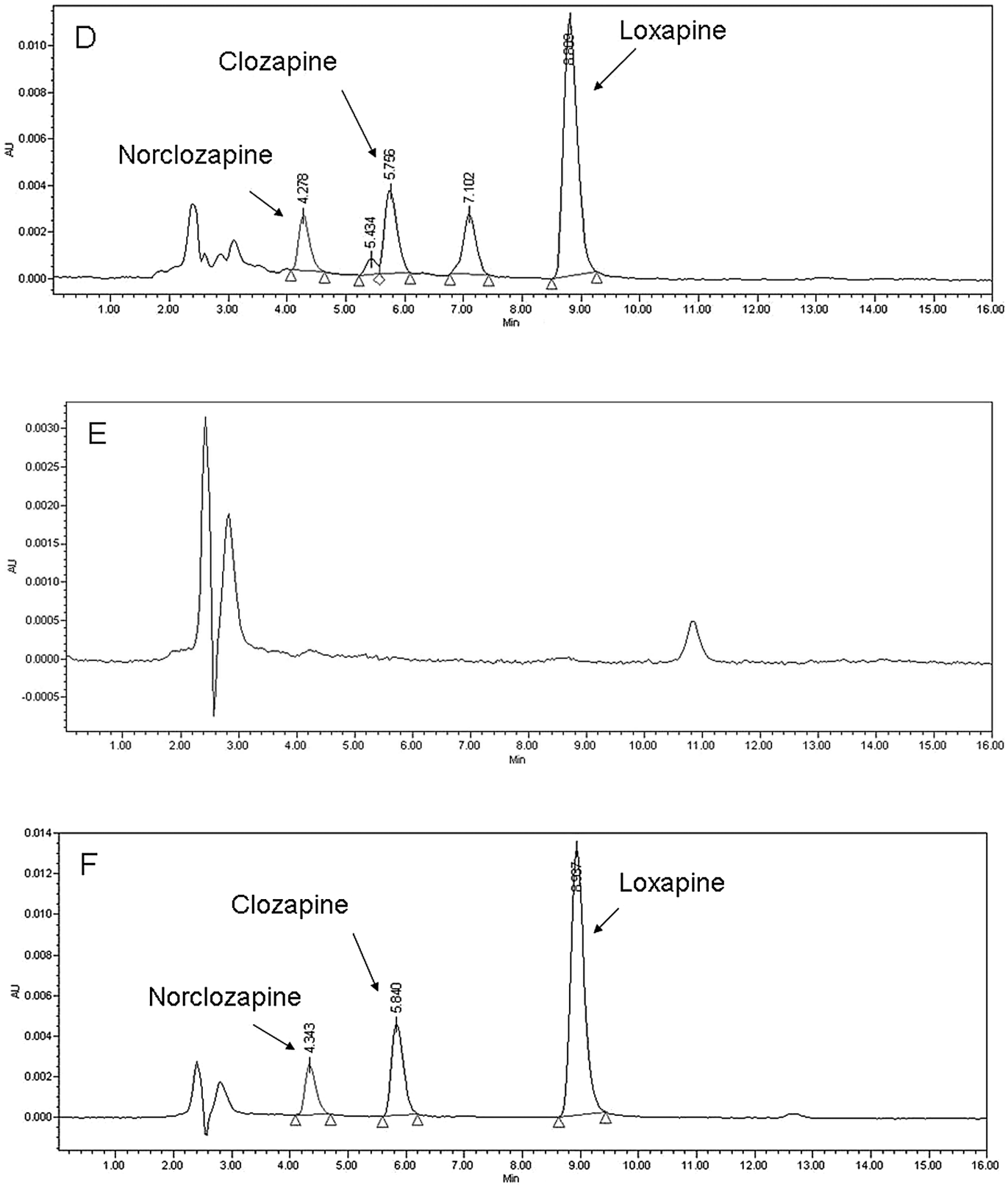

FIGURE 2. (*Continued*).

TABLE 1
Interday Reproducibility in Human Plasma

Clozapine (n = 6)	LLOQ (100 ng/mL)	LQC (300 ng/mL)	MQC (450 ng/mL)	HQC (900 ng/mL)
Average	103.00	293.56	454.35	921.74
% CV	9%	6%	8%	7%
% Accuracy	103%	98%	101%	102%
Norclozapine (n = 6)	LLOQ (50 ng/mL)	LQC (150 ng/mL)	MQC (300 ng/mL)	HQC (900 ng/mL)
Average	49.65	145.73	303.37	932.45
% CV	7%	10%	6%	4%
% Accuracy	99%	97%	101%	104%

Extractions were done on 6 different days with each QC extracted in triplicate. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

TABLE 2
Intraday Reproducibility

Clozapine (n = 6)	LLOQ (100 ng/mL)	LQC (300 ng/mL)	MQC (450 ng/mL)	HQC (900 ng/mL)
Average	108.27	325.65	510.71	954.90
% CV	8%	2%	6%	6%
% Accuracy	108%	109%	113%	106%
Norclozapine (n = 6)	LLOQ (50 ng/mL)	LQC (150 ng/mL)	MQC (300 ng/mL)	HQC (900 ng/mL)
Average	46.60	169.78	329.32	996.33
% CV	5%	2%	7%	7%
% Accuracy	93%	113%	110%	111%

Extraction was done on 1 day with each 6 of each QC. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

TABLE 3
Extraction Efficiency from Human Plasma

	LQC	MQC	HQC
% Extraction Efficiency Clozapine	101%	107%	103%
% Extraction Efficiency Norclozapine	89%	94%	96%

For each analyte, 6 extracted QC samples were compared with 6 non extracted QC samples. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

(Table 3). Extraction efficiencies greater than 100% were determined to be due to experimental error. Autosampler stability was determined by extracting 3 of each QC sample from human plasma and storing the reconstituted sample in the autosampler at 10°C for 80 h. The samples were then compared to a freshly extracted calibration curve. Clozapine autosampler stability accuracy was determined to be 94 to 107% and precision of 1 to 9%. Norclozapine autosampler stability accuracy was determined to be 94 to 112% and precision of 4 to 8% (Table 4). Bench top stability was determined by preparing 3 of each QC sample in human plasma and storing them on the bench at room temperature for 2 h prior to extraction. The samples were compared to a freshly extracted calibration curve. Clozapine bench top stability accuracy was determined to be 99 to 108% and precision of 0 to 2%. Norclozapine bench top stability accuracy was determined to be 89 to 104% and precision of 3 to 7% (Table 5).

Alternative matrices were partially validated by using the same extraction and analytical conditions and determining intraday precision and accuracy. For each matrix, a calibration curve and 3 of each QC samples were extracted and accuracy and precision were determined as above. Clozapine accuracy was 92 to 113% and precision was 1 to 7% in rat plasma, TRL,

TABLE 4
Sample Stability in the Autosampler

Clozapine (n = 3)	LLOQ (100 ng/mL)	LQC (300 ng/mL)	MQC (450 ng/mL)	HQC (900 ng/mL)
Average	107.01	281.72	422.04	892.81
% CV	6%	4%	9%	1%
% Accuracy	107%	94%	94%	99%
Norclozapine (n = 3)	LLOQ (50 ng/mL)	LQC (150 ng/mL)	MQC (300 ng/mL)	HQC (900 ng/mL)
Average	56.07	140.50	299.99	932.46
% CV	3%	5%	8%	4%
% Accuracy	112%	94%	100%	104%

Three of each QC was extracted from human plasma and stored in the autosampler for 80 h at 10ºC before analyzed. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

TABLE 5
Benchtop Stability in Human Plasma

Clozapine (n = 3)	LLOQ (100 ng/mL)	LQC (300 ng/mL)	MQC (450 ng/mL)	HQC (900 ng/mL)
Average	107.85	296.07	458.80	901.62
% CV	1%	0%	2%	1%
% Accuracy	108%	99%	102%	100%
Norclozapine (n = 3)	LLOQ (50 ng/mL)	LQC (150 ng/mL)	MQC (300 ng/mL)	HQC (900 ng/mL)
Average	44.40	150.11	312.47	913.75
% CV	7%	7%	4%	3%
% Accuracy	89%	100%	104%	102%

Three of each QC were extracted from human plasma after clozapine and norclozapine incubated in matrix for 2 h on the bench top. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

LDL, HDL, and LPDP human lipoprotein plasma fractions. Norclozapine accuracy was 88 to 116% and precision was 1 to 9% in rat plasma, TRL, LDL, HDL, and LPDP human lipoprotein plasma fractions (Table 6).

DISCUSSION

The results show an accurate and reproducible analytical method by HPLC for the quantitation of clozapine and its metabolite norclozapine, in human plasma. The calibration curves were with in the linear dynamic range of the assay for both analytes. Both interday and intraday determinations show less than 10% coefficient of variation and accuracy within ± 20% of nominal concentration for the quality control samples. We were able to show stable extraction efficiency across the linear range was near 100% for both analytes and that extracted samples were stable in the autosampler for reanalysis if required. We found the chromatography was sensitive to temperature and pH changes and could be a source of problems when trying to separate analytes from matrix peaks. Although our work shows clozapine and norclozapine are stable in human plasma for up to 2 h on the bench, other validations have shown clozapine and norclozapine could be stable in matrix for up to 3 days (Mosier et al., 2003).

The partial validation for the rat and various human plasma lipoprotein fractions showed responses very similar to that of the extraction of human plasma. Linearity of the calibration curves, calculated concentrations of calibration points and quality control samples were the similar to that in the human plasma matrix. As the change in the matrix from human plasma to rat plasma and human plasma lipoprotein fractions did not appear to change the ability of the extraction to remove clozapine and norclozapine or produce any significant matrix induced interference, we feel confident this assay can be applied to these additional matrices and have full confidence in the data obtained as though full validations were performed.

Future studies in the lipoprotein fractions should include a cross validation to radiolabeled clozapine and norclozapine. The process of validating a HPLC method requires the addition of analytes to blank matrix followed by their extraction, the addition of analyte to separated lipoprotein fractions may not be analogous to plasma containing analytes and then separated in to the various lipoprotein fractions. This issue cannot be resolved by any direct HPLC experiment and can only be confirmed by comparison to radiolabeled analytes.

TABLE 6
Partial Validation of Rat Plasma and Human Plasma Lipoprotein Fractions

	Clozapine				Norclozapine			
Matrix	LLOQ (100 ng/mL)	LQC (300 ng/mL)	MQC (450 ng/mL)	HQC (900 ng/mL)	LLOQ (50 ng/mL)	LQC (150 ng/mL)	MQC (300 ng/mL)	HQC (900 ng/mL)
Rat Plasma								
Average	106.44	289.10	447.30	902.64	43.95	155.54	300.36	952.67
% CV	5%	1%	2%	4%	9%	2%	4%	5%
% Accuracy	106%	96%	99%	100%	88%	104%	100%	106%
TRL Fraction								
Average	107.59	304.04	483.20	921.54	45.65	159.13	320.71	956.33
% CV	1%	4%	7%	3%	3%	3%	6%	4%
% Accuracy	108%	101%	107%	102%	91%	106%	107%	106%
HDL Fraction								
Average	113.37	318.78	496.35	988.13	43.77	159.88	334.29	1045.76
% CV	4%	1%	3%	2%	4%	5%	2%	3%
% Accuracy	113%	106%	110%	110%	88%	107%	111%	116%
LDL Fraction								
Average	107.69	308.11	470.08	934.19	43.19	159.01	307.27	947.80
% CV	2%	3%	4%	1%	9%	4%	4%	1%
% Accuracy	108%	103%	104%	104%	86%	106%	102%	105%
LPDP Fraction								
Average	92.01	310.61	452.20	937.15	49.16	146.19	317.35	865.09
% CV	7%	7%	2%	2%	2%	5%	5%	6%
% Accuracy	92%	104%	100%	104%	98%	97%	106%	96%

Three of each QC sample were extracted with each matrix. TRL—Human triglyceride rich lipoprotein fraction, HDL—Human high density lipoprotein fraction, LDL—Human low density lipoprotein fraction, LPDP—Human lipoprotein deficient plasma fraction. LLOQ—Lower Limit of Quantitation, LQC—Low Quality Control, MQC—Medium Quality Control, HQC—High Quality Control.

In summary we have shown a fast and easy extraction of clozapine and norclozapine in various matrices. All of the matrices show similar accuracy and precision and no interferences in the chromatography. If lower detection limits are required, a transfer to a LC/MS or LC/MS-MS could be done as all solvents and flow are MS compatible.

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REFERENCES

Almeida, A. M., Castel-Branco, M. M. et al. (2002). Linear regression for calibration lines revisited: Weighting schemes for bioanalytical methods. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, *774*(2), 215–222.

Ananth, J., Parameswaran, S., & Hara, B. (2004). Drug therapy in schizophrenia. *Curr. Pharm. Des.*, *10*(18), 2205–2217.

Byerly, M. J., & DeVane, C. L. (1996). Pharmacokinetics of clozapine and resperidone: A review of recent literature. *J. Clin. Psychopharmacol.*, *16*(2), 177–187.

Honer, W. G., Thornton, A. E., Chen, E. Y. H., Chan, R. C. K., Wong, J. O. Y., Bergmann, A., Falkai, P., Pomarol-Clotet, E., McKenna, P. J., Stip, E., Williams, R., MacEwen, G. W., Wasan, K. M., Procyshyn, R. (2006). Clozapine alone versus clozapine and resperidone with refractory schizophrenia. *N. Eng. l J. Med.*, *354*(5), 472–482.

Horacek, J., Bubenikova-Valesova, V., Kopecek, M., Palenicek, T., Dockery, C., Mohr, P., & Hoschi, C. (2006). Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. *CNS Drugs*, *20*(5), 389–409.

Khan, A. Y., & Preskorn, S. H. (2005). Examining concentration-dependant toxicity of clozapine: Role of therapeutic drug monitoring. *J. Psychiatr. Pract.*, *11*(5), 289–301.

Kuppamaki, M., Syvalahti, E., & Hietala, J. (1993). Clozapine and *N*-desmethylclozapine are potent 5-HT1C receptor antagonists. *Eur. J. Pharmcol.*, *245*(2), 179–182.

Procyshyn, R. M., Ho, T., Wasan, K. M. (2005). Competitive displacement of clozapine from plasma proteins in normolipidimic and hyperlipidimic plasma samples: Clinical implications. *Drug Dev. Ind. Pharm.*, *31*(4–5), 331–337.

Procyshyn, R. M., Kennedy, N. B., Marriage, S., & Wasan, K. M. (2001). Plasma protein and lipoprotein distribution of clozapine. *Am. J. Psychiatry.*, *158*(6), 949–951.

Ma, F., & Lau, C. E. (1998). Determination of clozapine and its metabolite, *N*-desmethylclozapine, in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats. *J. Chromatogr. B Biomed. Sci. Appl.*, *712*(1–2), 193–198.

Mosier, K. E., Song, J., McKay, G., Hubbard, J. W., Fang, J. (2003). Determination of clozapine, and it metabolites, *N*-desmethylclozapine and clozapine N-oxide in dog plasma using high-performance liquid chromatography. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, *783*(2), 377–382.

Wasan, K. M., Cassidy, S. M., Ramaswamy, M., Kennedy, A., Strobel, F. W., Ng, S. P., & Lee, T. Y. (1999). A comparison of step-gradient and sequential density ultracentrifugation and the use of lipoprotein deficient plasma controls in determining the plasma lipoprotein distribution of lipid associated nystatin and cyclosporine. *Pharm. Res.*, *16*(1), 165–169.