UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS

PROGRAMA DE POSGRADO EN CIENCIAS FARMACOBIOLÓGICAS

"FARMACOCINÉTICA POBLACIONAL Y FARMACOGENÉTICA DE SERTRALINA EN PACIENTES CON PATOLOGÍA DUAL"

T E S I S

QUE PARA OBTENER EL TÍTULO DE:

DOCTORA EN CIENCIAS FARMACOBIOLÓGICAS

PRESENTA:

M.C. CINTHYA ELOISA CHÁVEZ CASTILLO

DIRECTOR DE TESIS:

DRA. SILVIA ROMANO MORENO

ASESORES:

DRA. ROSA DEL CARMEN MILÁN SEGOVIA

DRA. SUSANNA EDITH MEDELLÍN GARIBAY

DR. SERGIO ZARAZÚA GUZMÁN

ASESOR EXTERNO:

DRA. HELGI HELENE JUNG COOK

ASESOR CLÍNICO:

PSIQ. MARISOL OROCIO CONTRERAS

SAN LUIS POTOSÍ, SLP. ENERO 2024

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Este proyecto se realizó en *Centros de Integración Juvenil A.C. de San Luis Potosí y en el Laboratorio de Farmacia* adscrito a la Facultad de Ciencias Químicas de la Universidad Autónoma de San Luis Potosí, en el periodo comprendido entre *agosto 2018 y agosto 2022*, bajo la dirección de la *Dra. Silvia Romano Moreno*.

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FARMACOCINÉTICA POBLACIONAL Y FARMACOGENÉTICA DE SERTRALINA EN PACIENTES CON PATOLOGÍA DUAL

Por Cinthya Eloisa Chavez Castillo

INFORME DE ORIGINALIDAD

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ÍNDICE DE SIMILITUD

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M.C. CINTHYA ELOISA CHÁVEZ CASTILLO

SINODALES

PRESIDENTE:
Dra. Rosa del Carmen Milán Segovia _____

SECRETARIO:
Dra. Silvia Romano Moreno _____

VOCAL:
Dra. Susanna Edith Medellín Garibay _____

VOCAL:
Dra. Helgi Helene Jung Cook _____

VOCAL:
Dr. Sergio Zarazúa Guzmán _____

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FACULTAD DE
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POSGRADO EN CIENCIAS FARMACOBIOLÓGICAS
Tel: 826-23-00 ext. 6541 y 6540

San Luis Potosí, S.L.P.
13 de diciembre de 2023

Comité Académico del Posgrado en Ciencias Farmacobiológicas
Facultad de Ciencias Químicas de UASLP
PRESENTE

Por medio de la presente comunicamos que la tesis llevada a cabo por la alumna de Doctorado **MC. Cinthya Eloisa Chávez Castillo**, titulada ***"Farmacocinética poblacional y farmacogenética de sertralina en pacientes con patología dual",*** ha sido concluida y aprobada por el comité tutorial para dar inicio a los trámites correspondientes para su titulación. El examen de grado tendrá lugar el próximo martes 16 de enero de 2024 a las 17:00 h en el Auditorio Chico (G203) de la Facultad.

Atentamente

Dra. Silvia Romano Moreno
Director de Tesis

Comité tutorial

Dra. Rosa del Carmen Milán Segovia
Asesor

Dra. Susanna Edith Medellín Garibay
Asesor

Dr. Sergio Zarazúa Guzmán
Asesor

Dra. Helgi Jung Cook
Asesor Externo

www.uaslp.mx

Av. Dr. Manuel Nava Núm. 6
Zona Universitaria · CP 78210
San Luis Potosí, S.L.P.
tel. (444) 826 24 40 al 46
fax (444) 826 2372

INTEGRANTES DEL SUBCOMITÉ DE TESIS

Director de tesis

Dra. Silvia Romano Moreno
Laboratorio de Farmacia. Facultad de Ciencias Químicas.
Universidad Autónoma de San Luis Potosí.

Asesor

Dra. Rosa del Carmen Milán Segovia
Laboratorio de Biofarmacia y Farmacocinética. Facultad de Ciencias Químicas.
Universidad Autónoma de San Luis Potosí.

Asesor

Dra. Susanna Edith Medellín Garibay
Laboratorio de Biofarmacia y Farmacocinética. Facultad de Ciencias Químicas.
Universidad Autónoma de San Luis Potosí.

Asesor

Dr. Sergio Zarazúa Guzmán
Laboratorio de Toxicología. Facultad de Ciencias Químicas.
Universidad Autónoma de San Luis Potosí.

Asesor externo

Dra. Helgi Helene Jung Cook
Laboratorio de Farmacia. Facultad de Ciencias Químicas.
Universidad Nacional Autónoma de México.

Asesor clínico

Psiq. Marisol Orocio Contreras
Centros de Integración Juvenil, A. C.
San Luis Potosí, México.

DEDICATORIA

A mi adorada familia, porque son lo más sagrado que tengo en la vida.
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Resumen

El objetivo de este estudio fue caracterizar la farmacocinética poblacional de sertralina en pacientes mexicanos con patología dual. Se incluyeron 59 pacientes (13 a 76 años) tratados con dosis de sertralina entre 12.5-100 mg/día. Se determinaron las concentraciones plasmáticas de sertralina en muestras de sangre y en muestras de orina se determinaron cinco de las principales sustancias de abuso mediante pruebas rápidas. También se evaluaron factores demográficos, clínicos y farmacogenéticos. El análisis farmacocinético poblacional se realizó mediante el software NONMEM® con el método de estimación condicional de primer orden. Las concentraciones de sertralina frente al tiempo se describieron adecuadamente por un modelo de un compartimento con error residual proporcional. El polimorfismo CYP2D6*2 y los fenotipos de CYP2C19 fueron las covariables que influyeron significativamente en el aclaramiento de sertralina, el cual tuvo un valor medio poblacional de 66 L/h en el modelo final. La constante de absorción y el volumen de distribución se fijaron en 0.855 1/h y 20.2 L/kg, respectivamente. El modelo explicó el 11.3 % de la variabilidad interindividual del aclaramiento de sertralina. La presencia del polimorfismo CYP2D6*2 causó una disminución del 23.1 % en el aclaramiento de sertralina, mientras que los pacientes con fenotipo intermedio y pobre de CYP2C19 mostraron disminuciones del 19.06 % y 48.26 % en el aclaramiento de sertralina, respectivamente. El modelo se validó internamente mediante bootstrap y visual predictive check. Finalmente, se realizaron simulaciones estocásticas para proponer regímenes de dosificación que permitan alcanzar niveles terapéuticos que contribuyan a mejorar la respuesta al tratamiento.

Palabras clave: Farmacocinética poblacional, sertralina, patología dual, CYP2D6, CYP2C19.

Abstract

The aim of this study was to characterize the population pharmacokinetics of sertraline in Mexican patients with dual pathology. Fifty-nine patients (13 to 76 years old) treated with doses of sertraline between 12.5 - 100 mg/day were included. Plasma sertraline concentrations were determined in blood samples and five of the main substances of abuse were determined by rapid tests in urine samples. Demographic, clinical and pharmacogenetic factors were also evaluated. Population pharmacokinetic analysis was performed using NONMEM® software with first order conditional estimation method. Sertraline concentrations versus time were adequately described by a one-compartment model with proportional residual error. CYP2D6*2 polymorphism and CYP2C19 phenotypes significantly influenced sertraline clearance which had a population mean value of 66 L/h in the final model. The absorption constant and volume of distribution were fixed at 0.855 1/h and 20.2 L/kg, respectively. The model explained 11.3 % of the interindividual variability in sertraline clearance. The presence of the CYP2D6*2 polymorphism caused a 23.1 % decrease in sertraline clearance, whereas patients with intermediate and poor phenotype of CYP2C19 showed 19.06 % and 48.26 % decreases in sertraline clearance, respectively. The model was internally validated by bootstrap and visual predictive check. Finally, stochastic simulations were performed to propose dosing regimens to achieve therapeutic levels that contribute to improve treatment response.

Keywords: Population pharmacokinetics; sertraline; dual pathology; CYP2D6; CYP2C19

TESIS EN FORMATO ARTÍCULO

ÍNDICE GENERAL

Farmacocinética poblacional y farmacogenética de sertralina en pacientes con patología dual

Introducción

La patología dual (PD) se define como la concurrencia en un mismo individuo de, por lo menos, un trastorno por uso de sustancias (TUS) y otro trastorno psiquiátrico (OTP). Se estima que en la población de los centros especializados de tratamiento, la prevalencia reportadas de PD va del 50 % al 75 %. Existen desafíos en el tratamiento farmacológico de pacientes con PD debido al incumplimiento durante el tratamiento, las altas tasas de recaída en el consumo de sustancias y rehospitalización, así como un mayor deterioro psicosocial debido a problemas legales, violencia y falta de apoyo comunitario. Además, aproximadamente el 40% de los pacientes tratados con inhibidores selectivos de la recaptación de serotonina (ISRS) no logran una buena respuesta al tratamiento.

Sertralina (SRT) es un antidepresivo ISRS que es utilizado en el tratamiento de trastorno de depresión mayor, trastorno obsesivo compulsivo, trastorno de ansiedad social (fobia social), trastorno de pánico y trastorno de estrés postraumático.

SRT alcanza concentraciones plasmáticas máximas 6 – 8 h post dosis, se une un 98 % a proteínas y tiene un volumen de distribución (Vd) de 20 L/kg. Se metaboliza en el hígado por diversas enzimas del CYP-450 que generan un metabolito activo, la N-desmetilsertralina (DSRT), el cual no tiene relevancia clínica. SRT presenta una cinética de eliminación de primer orden con una vida media de eliminación ($t_{1/2}$) de 26 h y de 60 a 100 h para DSRT.

La farmacocinética de SRT muestra una alta variabilidad que puede deberse a factores como la edad, el género, la función hepática, la comedicación con metamizol, el jugo de toronja y polimorfismos en CYP2C19 y CYP2B6.

Las dosis de SRT en PD son determinadas con base a guías de práctica clínica, que, si bien brindan una orientación, no aseguran alcanzar los niveles plasmáticos dentro del intervalo terapéutico de 10 a 150 ng/mL. Por lo que, el desarrollo de un modelo de

farmacocinética poblacional de SRT que permita establecer regímenes de dosificación en pacientes con PD, podría tener un impacto importante, ya que permitiría garantizar la eficacia del tratamiento farmacológico, minimizando la probabilidad de la aparición de efectos adversos.

Objetivos

Objetivo general

Desarrollar y validar un modelo farmacocinético poblacional para SRT en pacientes mexicanos con PD con el fin de establecer regímenes de dosificación confiables y adaptados a las características antropométricas, fisiopatológicas, de comedicación, genéticas y de consumo de sustancias de cada paciente.

Objetivos específicos

- Implementar y validar un método analítico de cromatografía de líquidos de ultra resolución acoplada a espectrometría de masas en tándem (UPLC-MS/MS) para la cuantificación de SRT en muestras plasmáticas de pacientes con PD.
- Determinar la presencia de las variantes genéticas CYP2D6*2, *4, *10, *17, *41, CYP2C9*2, *3, CYP2C19*2, *17 y CYP2B6*9.
- Evaluar la influencia de factores no genéticos y genéticos de la población en estudio en la farmacocinética de SRT en pacientes con PD mediante la aplicación de modelaje de efectos mixtos empleando el programa NONMEM®.
- Validar internamente el modelo farmacocinético poblacional obtenido mediante la predicción *"a priori"* de las concentraciones plasmáticas de SRT en pacientes y mediante la técnica de remuestreo (Bootstrap).
- Diseñar pautas de dosificación para SRT adaptadas a las características de cada paciente de acuerdo con el modelo farmacocinético poblacional obtenido.

Material y métodos

El protocolo de investigación de este estudio fue aprobado por el Comité de Investigación Científica de Centros de Integración Juvenil (CIJ) (número de registro:

203-19) y por el Comité de Ética en Investigación y Docencia de la Facultad de Ciencias Químicas de la Universidad Autónoma de San Luis Potosí (número de registro: CEID2019-09-S).

Este fue un estudio analítico, observacional, prospectivo y longitudinal. La inclusión de los pacientes se realizó en el CIJ de San Luis Potosí. Se incluyeron pacientes ambulatorios con diagnóstico de PD mayores de 13 años que estuvieran en tratamiento con SRT. Todos los pacientes mayores de 18 años firmaron el consentimiento informado; en el caso de menores de edad, se solicitó la firma del consentimiento informado por parte de alguno de los padres y se solicitó la firma del asentimiento informado por parte del menor.

Implementación y validación del método analítico de UPLC-MS/MS para la cuantificación de SRT en plasma.

Para la cuantificación de SRT se desarrolló un método analítico de Cromatografía de Líquidos de Ultra Resolución acoplada a espectrometría de masas en tándem (UPLC-MS/MS).

La separación cromatográfica se llevó a cabo en una columna Acquity UPLC BEH C18 de 2.1 x 50 mm, 1.7 µm (Waters, Corp.) y una precolumna VanGuard® (Waters, Corp.) de 2.1 mm x 5 mm, 1.7 µm mediante un sistema ACQUITY UPLC-Clase H acoplado al módulo de espectrometría de masas en tándem XEVO-TQD (Waters, Corp.).

Se utilizó una fase móvil de acetato de amonio 5 mM con 0.1 % de ácido fórmico y acetonitrilo (60:40 % v/v) a un flujo fue de 0.4 mL/min con elución isocrática.

El procesamiento de las muestras se realizó a partir de 200 µL a los cuales se le añadieron 400 µL de acetonitrilo que contenía el estándar interno de indometacina (IND) a una concentración final de 100 ng/mL. La mezcla se centrifugó y se tomó una alícuota de 450 µL que se evaporó hasta sequedad. Posteriormente, el residuo se reconstituyó con 100 µL de fase móvil, la mezcla fue centrifugada y después se transfirió el sobrenadante a un vial de vidrio.

El volumen de inyección fue de 20 µL y el tiempo de corrida 5 min. Para la cuantificación de SRT se utilizó la transición (*m/z*) 308.1 > 160.90 con un voltaje de

cono de 20 V y una energía de colisión de 24 V. Por otra parte, para la cuantificación de IND se utilizó la transición (*m/z*) 358.08 > 138.08, con un voltaje de cono de 34 V y unan energía de colisión de 18 V.

La validación del método analítico se realizó de acuerdo con los parámetros establecidos en la NOM-177-SSA1-2013.

Obtención de muestras plasmáticas y de orina de pacientes con PD.

Una vez iniciado el tratamiento con SRT se tomó una muestra de sangre y una muestra de orina al menos 7 días después del inicio del tratamiento. En otra visita posterior, se tomó una segunda muestra de sangre y orina, al menos 30 días después de la primera muestra.

Las muestras de sangre se recolectaron en tubos Vacutainer® con EDTA, después fueron centrifugadas a 1300 rpm por 20 min y el plasma fue almacenado a -80 °C hasta su análisis. Las muestras de orina fueron empleadas para realizar una prueba rápida multidrogas (Instant View®) para determinar marihuana, anfetaminas, metanfetaminas, cocaína y benzodiacepinas.

En cada uno de los muestreos se realizó una entrevista con el paciente para obtener información sobre su tratamiento, consumo de sustancias y también se le aplicaron la Escala de Beck para ansiedad (BAI) y la Escala de Beck para depresión (BDI). Del expediente clínico de cada paciente se obtuvo información sobre variables antropométricas, diagnóstico, sustancias consumidas, duración de tratamiento y parámetros bioquímicos como glucosa, colesterol total, triglicéridos, aspartato aminotransferasa (AST), alanina transaminasa (ALT), creatinina sérica, hemoglobina, hematocrito y plaquetas.

Genotipificación.

A partir de una muestra sanguínea del paciente, se obtuvo ADN genómico (DNA Preparation kit® de Jena Bioscience) para determinar los polimorfismos CYP2D6*2, *4, *10, *17, *41, CYP2C9*2, *3, CYP2C19*2, *17, CYP2B6*9 mediante PCR tiempo real con el empleo de sondas TaqMan®.

Desarrollo y validación del modelo farmacocinético poblacional.

El modelo farmacocinético poblacional se desarrolló con el software farmacoestadístico NONMEM v.7.4. Se evaluaron modelos de uno y dos compartimentos con absorción y eliminación de primer orden mediante las subrutinas ADVAN2 TRANS2 y ADVAN4 TRANS4, respectivamente, mediante el método de estimación FOCE (First Order Conditional Estimation).

Se evaluaron modelos de error exponencial y proporcional para la variabilidad interindividual (VII) y modelos de error aditivo, proporcional y combinado para la variabilidad residual (VR).

La elección entre los distintos modelos obtenidos se basó en el valor mínimo de la función objetivo (OFV), los gráficos de predicción del modelo y la plausibilidad de los parámetros.

Los valores iniciales de los parámetros farmacocinéticos como constante de absorción (Ka), volumen de distribución (Vd) y aclaramiento (CL) se tomaron de estudios farmacocinéticos previos.

Una vez que se desarrolló el modelo base, se analizó el efecto de cada una de las covariables continuas en el modelo, mediante funciones lineales, alométricas o exponenciales. Posteriormente se evaluó la influencia de cada una de las variables categóricas para obtener el modelo completo.

Las variables continuas analizadas fueron: edad, peso, estatura, glucosa sérica, urea, triglicéridos, colesterol total, creatinina, estimación de Crockroft-Gault del aclaramiento de creatinina, albúmina, AST, ALT, hemoglobina, hematocrito, plaquetas, índice de masa corporal, las puntuaciones BAI y BDI. Asimismo, se analizaron las siguientes covariables categóricas: sexo, consumo de tabaco, alcohol y jugo de toronja, comorbilidades, comedicación, genotipos, fenotipos y los resultados de las pruebas rápidas multidrogas en orina.

Para definir las covariables incluidas en el modelo completo, se consideró una disminución de al menos 3.84 unidades de la OFV ($p < 0.05$) al añadir una covariable.

Posteriormente, para definir el modelo final se consideró un criterio estadístico más estricto para las variables cuya eliminación del modelo completo produjera un aumento de al menos 6.63 unidades de la OFV ($p < 0.01$).

La precisión y estabilidad del modelo final se evaluó internamente mediante bootstrap con 1000 réplicas, se calcularon la mediana y los intervalos de confianza al 95 % de los parámetros farmacocinéticos. Asimismo, se realizó una evaluación visual predictiva (VPC) para analizar la capacidad de predicción del modelo.

Simulaciones estocásticas para el desarrollo de regímenes de dosificación de SRT.

Con el modelo poblacional final, se realizaron 200 simulaciones estocásticas de las concentraciones mínimas de SRT para proponer regímenes de dosificación.

Resultados y discusión

El método analítico desarrollado y validado fue lineal en un intervalo de 5 a 800 ng/mL ($R^2 > 0.991$) con porcentajes de recobro de SRT en plasma entre el 92 – 106 %. El método fue preciso y exacto ya que en el análisis de repetibilidad se tuvieron coeficientes de variación del 3.10 % al 10.69 %, mientras que, para el análisis de la reproducibilidad, se tuvieron valores en el rango de 4.96 % al 11.11 %. De igual manera, se tuvieron % de desviación < 15 % para las muestras control y < 20% para las muestras del límite inferior de cuantificación.

Además, se demostró que SRT fue estable en plasma después de tres ciclos de congelación-descongelación y en condiciones de almacenamiento (-80 °C) hasta por 30 días, con porcentajes de desviación de 5.82 % y 5.86 %, respectivamente.

Se incluyeron 59 pacientes con PD (55.9 % hombres) tratados con SRT, de los cuales se obtuvieron un total de 75 muestras plasmáticas. La edad de los pacientes tuvo un rango entre los 13 – 76 años, con un peso y altura promedio de 71.9 kg y 1.70 m, respectivamente. La dosis promedio de SRT fue 50 mg/día, mientras que la concentración plasmática media de SRT fue 44.46 ng/mL.

Los TUS más frecuentes fueron abuso o dependencia de alcohol (69.5 %), dependencia de nicotina (32.2 %), abuso o dependencia de anfetaminas (27.1 %), abuso o dependencia de marihuana (22.0 %), abuso de inhalantes (5.1 %) y dependencia de cocaína (6.8 %).

Los OTP que presentaban los pacientes eran trastorno depresivo mayor (37.3 %), trastorno de ansiedad (33.9 %), trastorno del estado de ánimo y de ansiedad inducido por anfetaminas (25.4 %), trastorno límite de la personalidad (20.3 %), trastorno del estado de ánimo y de ansiedad inducido por alcohol (16.9 %) y trastorno depresivo persistente (15.3 %), trastorno de la personalidad por dependencia (8.5 %), trastorno psicótico con alucinaciones inducido por anfetaminas (8.5 %) y trastorno por déficit de atención con hiperactividad (5.1 %).

En cuanto a los resultados positivos de las pruebas rápidas multidroga en orina, 18 (24.0 %) fueron para marihuana, 8 (10.7 %) para metanfetaminas, 5 (6.7 %) para anfetaminas, 5 (6.7 %) para benzodiacepinas y 2 (2.7 %) para cocaína.

Los principales medicamentos concomitantes fueron antipsicóticos (20.0 %), antihipertensivos (17.3 %), antiepilépticos (14.7 %), ansiolíticos (13.3 %), antiulcerosos (5.3 %), antiinflamatorios no esteroideos (6.7 %) y antidiabéticos (6.7 %). Mientras que las principales comorbilidades fueron hipertensión (18.6 %), epilepsia (13.6 %), diabetes mellitus tipo II (10.2 %) e hipotiroidismo (68 %).

El modelo abierto de un compartimento con absorción y eliminación de primer orden fue el que mejor describió las concentraciones plasmáticas de SRT contra el tiempo. El único parámetro farmacocinético estimado fue el CL de SRT, mientras que la constante de absorción (Ka) se fijó en 0.855 1/h y el volumen de distribución (Vd) se fijó en 20.2 L/kg. La variabilidad interindividual (VII) se modeló exponencialmente, mientras que para la variabilidad residual (VR) se eligió un modelo de error proporcional.

En el modelo base, el CL de SRT se estimó en 57.1 L/h con una VII de 38.2 % y una VR de 0.29 %. Tras la evaluación de la influencia de cada covariable en la farmacocinética de SRT, las covariables que demostraron tener un efecto

estadísticamente significativo ($p < 0.01$) sobre el CL fueron CYP2D6*2 y el fenotipo de CYP2C19.

En el modelo final, el CL de SRT se estimó en 66 L/h y permitió explicar el 11.3 % de la VII en el CL de SRT. Dicho valor para el CL de SRT está en concordancia con los valores reportados para este parámetro en estudios farmacocinéticos poblacionales previos (36.3 – 130 L/h). Por otra parte, no se observó disminución de la VR de las concentraciones de SRT, sin embargo, el valor obtenido (0.31 %) es mucho menor a valores reportados en estudios previos (11.70 - 48.47%).

En nuestro estudio, la presencia del polimorfismo CYP2D6*2 causó una disminución del 23.1 % en el CL de SRT con respecto a pacientes con el genotipo de referencia. La frecuencia alélica observada fue 26.3 %, la cual es muy similar a frecuencias reportadas para población mexicana mestiza (19.34 %), mexicanoamericana (18 – 22.8 %), española (22 %) y caucásica (28.5 %).

Los fenotipos de CYP2C19 que se identificaron en los pacientes fueron el metabolizador normal (MN) (74.6 %), el intermedio (MI) (11.9 %), el rápido (MR) (11.9 %) y el pobre (MP) (1.7 %). Para facilitar el análisis farmacocinético, los pacientes MR se consideraron dentro del grupo MN, ya que los valores de CL de estos grupos fueron muy similares y no mostraron diferencias significativas ($p = 0.354$).

Los pacientes con fenotipo MI mostraron una disminución del 19.06 % en el CL de SRT, mientras que, los pacientes MP mostraron una disminución del 48.26 % en el CL de SRT con respecto a los pacientes MN. Estos resultados son similares a los previamente reportados que indican que los MI presentan disminuciones entre el 14.95 % - 17.84 % y los MP presentan disminuciones entre 29.04 % - 43.87 % en el CL de SRT.

El modelo final se validó internamente mediante remuestreo (bootstrap). Los resultados obtenidos demostraron una precisión adecuada, ya que los parámetros estimados a partir del modelo final se encontraron dentro de los percentiles 2.5 y 97.5. Así mismo, a partir de los resultados del visual predictive check (VPC) se demostró que la variabilidad se estimó adecuadamente en el modelo, ya que la mayoría de las concentraciones de SRT se encontraban dentro de las regiones de confianza

predichas por el modelo (intervalo de confianza del 90 %) para la mediana y los percentiles 5 y 95.

A partir del modelo obtenido, se realizaron simulaciones de las concentraciones plasmáticas mínimas de SRT tomando en cuenta las variables que influyeron en el CL de SRT. Inicialmente, se simuló una dosis estándar de 50 mg/día para cada uno de los seis grupos de tratamiento establecidos: 1) CYP2D6*2 + PM, 2) PM, 3) CYP2D6*2 + IM, 4) IM, 5) CYP2D6*2 + EM y 6) EM.

Posteriormente se simularon dosis de 75, 100, 150 y 200 mg/día, con lo cual se pudieron determinar los regímenes de dosificación más adecuados para cada uno de los grupos de tratamiento.

Para los fenotipos de CYP2C19, se determinó que para los pacientes MN la dosis recomendada es 200 mg/día de SRT, para los MI 150 mg/día y para los MP 75 – 100 mg/día. Sin embargo, cuando también se toma en cuenta la presencia del polimorfismo CYP2D6*2 además del fenotipo de CYP2C19, las dosis de SRT deben reducirse.

Para pacientes MN con polimorfismo CYP2D6*2 la dosis recomendada es de 150 mg/día, para los pacientes MI con polimorfismo CYP2D6*2 la dosis recomendada es de 75 – 100 mg/día y para los MP con polimorfismo CYP2D6*2 la dosis recomendada es de 50 – 75 mg/día.

La efectividad en la práctica clínica de los regímenes de dosificación de SRT propuestos deberán ser validados externamente en una población similar, por lo que deberán realizarse estudios prospectivos adicionales para evaluar su impacto en la mejoría de la respuesta al tratamiento con SRT.

Este es el primer estudio que describe el comportamiento farmacocinético de SRT en pacientes mexicanos con patología dual. Los resultados aquí presentados se describen en el artículo *“Population pharmacokinetics of sertraline in psychiatric and substance use disorders”* enviado para su publicación a la revista *The Journal of Clinical Pharmacology* (Anexo II).

Conclusiones

Se desarrolló y validó un modelo farmacocinético de SRT en pacientes con PD que demostró una precisión adecuada en la estimación de las concentraciones mínimas de SRT. Este es el primer estudio que caracteriza la influencia tanto del polimorfismo CYP2D6*2 como los fenotipos MI y MP de CYP2C19 en el CL de SRT. Los regímenes de dosificación propuestos se establecieron tomando en cuenta estas covariables, con el objetivo de que los pacientes puedan alcanzar niveles terapéuticos que contribuyan a mejorar la respuesta al tratamiento.

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Artículos de investigación

Anexo I.

Simultaneous determination of four serotonin selective reuptake inhibitors by an UPLC MS-MS method with clinical application in therapeutic drug monitoring

Autores:

Cinthya Eloisa Chávez-Castillo, Julia Sagahón-Azúa, Karla Itzel Velasco-Gloria, Susanna Edith Medellín-Garibay, Rosa del Carmen Milán-Segovia, Silvia Romano-Moreno

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Simultaneous determination of four serotonin selective reuptake inhibitors by an UPLC MS-MS method with clinical application in therapeutic drug monitoring

Cinthya Eloisa Chávez-Castillo, Julia Sagahón-Azúa, Karla Itzel Velasco-Gloria, Susanna Edith Medellín-Garibay, Rosa del Carmen Milán-Segovia, Silvia Romano-Moreno *

Pharmacy Department, Faculty of Chemical Sciences, Autonomous University of San Luis Potosi, S.L.P, Mexico

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ABSTRACT

An analytical method of ultra-high performance liquid chromatography coupled to tandem mass spectrometry detection was developed and validated for the simultaneous quantification in plasma of four selective serotonin reuptake inhibitor antidepressants: sertraline, escitalopram, paroxetine, fluoxetine, and its metabolite norfluoxetine. A simple protein precipitation was performed with acetonitrile containing 100 ng/mL of indomethacin, which was used as internal standard. Chromatographic separation was carried out on an Acquity BEH C18 column with isocratic elution of the mobile phase consisting of 5 mmol/L ammonium acetate with 0.1% formic acid (A) and acetonitrile (B) at a 60:40 proportion, respectively. The flow rate was 0.4 mL/min with a run time of 5 min. A positive electrospray ionization source was used for detection. The method was linear in a range of 5-800 ng/mL, with determination coefficients greater than 0.991. The accuracy ranged from 91% to 112% for intra-assay and from 89% to 112% for inter-assay. The variation coefficients ranged from 3.1% to 14.88% for intra-assay and from 3.60% to 14.74% for inter-assay precision. The method was successfully applied for the analysis of 73 samples from patients under treatment with these antidepressants; 36.9% of the samples had concentrations outside therapeutic ranges. This method can be applied for routine analysis in clinical practice, simplifying sample processing, reducing analysis time and consequently the costs associated with it.

1. Introduction

Selective serotonin reuptake inhibitors (SSRI) were the first class of rationally designed drugs in psychiatry. Nowadays, they are known as second generation antidepressants [1] and these are the first-line option in the pharmacological treatment of major depression [2]. The introduction of fluvoxamine (FLV) was followed by fluoxetine (FLX), paroxetine (PRX), citalopram (CIT), sertraline (SRT) and escitalopram (ESC) [3].

All SSRI inhibit the neuronal reuptake of serotonin, which increases the synaptic availability of this neurotransmitter [1,4]. However, they differ in their chemical structures (Fig. 1), metabolism, pharmacokinetics, and the inhibitory effect on the cytochrome *P*-450 system, resulting in different clinical profiles [5,6].

Therapeutic drug monitoring (TDM) is a tool that optimizes drugs dosing based on the quantification of blood concentrations and individual patient́s characteristics [7]. SSRI are drugs that exhibit wide interindividual pharmacokinetic variability [8], whereby TDM is useful for compliance monitoring, confirmation of sub-therapeutic or toxic concentrations and monitoring after overdose [9].

The Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP), an interdisciplinary TDM group, proposed guidelines for therapeutic reference ranges in which the maximal efficacy is achieved with acceptable safety for SSRI: FLV (60–230 ng/mL), PRX (20–65 ng/mL), SRT (10–150 ng/mL), ESC (15–80 ng/mL), CIT (50–110 ng/mL), FLX plus its main active metabolite norfluoxetine (NFLX) (120–500 ng/mL) [10].

To determine SSRI concentrations, highly sensitive and specific analytical methods are required in which interferences from other drugs or endogenous components are minimal [11]. Liquid chromatography (LC) is the most widely accepted technique for the determination of SSRI and their metabolites.

* Corresponding author at: Pharmacy Department, Faculty of Chemical Sciences, Autonomous University of San Luis Potosi, 6 Dr. Manuel Nava Avenue, San Luis Potosi 78210, Mexico.
E-mail address: srm@uaslp.mx (S. Romano-Moreno).

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A significant number of methods have been developed for the simultaneous determination of SSRI and their metabolites in human plasma and serum samples by high-performance liquid chromatography (HPLC) coupled to ultraviolet detector [12], HPLC coupled to diode array detector [13,14], HPLC coupled to mass spectrometry [15], HPLC coupled to tandem mass spectrometry (MS/MS) [16–19] and recently ultra-high performance liquid chromatography (UPLC)-MS/MS methods have also been proposed [20–24].

For sample processing, several techniques have been used such as solid phase extraction [12,15,18,19,25], liquid–liquid extraction [13,14,17,21,22] and protein precipitation (PPT) with methanol and formic acid [23], zinc sulfate in acetonitrile/methanol [20] and acetonitrile [24], which have been shown to be effective for the simultaneous isolation of multiple drugs, mainly antidepressants and antipsychotics, in plasma and serum samples.

Various simultaneous methods to determine SSRI used stable isotopes labeled as internal standard (IS) [18,19,21,22,24], which contribute to minimize the influence of matrix effects on analyte quantification [26]. However, these reagents are expensive, which increases quantification costs and not all laboratories have the opportunity or budget to access them.

An alternative approach has been reported in several methods in which a drug was chosen as IS for simultaneous quantification of SSRI [12–15,20,23], however, the main disadvantage of this approach is the possible interference with the method if the patient takes the drug used as IS. Nevertheless, with the appropriate chromatographic conditions, cost-effective analytical methods can be developed and applied in clinical practice to quantify SSRI with adequate values of sensitivity, accuracy and precision.

Therefore, the aim of this work was to develop and validate an UPLC-MS/MS method for the simultaneous quantification in plasma of SRT, PRX, ESC, FLX and their main metabolite NFLX with a simple and low-cost sample processing method that can be applied clinically for TDM.

2. Methodology

2.1. Chemicals and biologicals

Certified analytical standards of SRT (>99.0%), PRX (97.5%), ESC (99.0%) FLX (99%) and the IS of indomethacin (IND, 99.7%) were purchased from Sigma Aldrich (State of Mexico, Mexico), NFLX (≥98%) was purchased from Cayman Chemical Company (Michigan, USA). Chemical structures of the analytes are shown in Fig. 1. Ammonium acetate (≥99.0%) and formic acid (98–100%) were purchased from Merck KGaA (Darmstadt, Germany). Acetonitrile LC-MS grade was purchased from Honeywell Burdick & Jackson (Muskegon, Michigan, USA). Methanol and acetonitrile HPLC grade were purchased from Fermont (Monterrey, Nuevo Leon, Mexico). The drug free plasma used to validate the method was obtained from the blood bank of the Central Hospital "Dr. Ignacio Morones Prieto" (CHIMP, San Luis Potosi, Mexico).

2.2. Stock and working solutions

Standard solutions of SRT, FLX, NFLX, ESC and PRX were prepared at an initial concentration of 1 mg/mL in methanol. Subsequently, a mixture of SSRI was prepared in methanol at a concentration of 10,000 ng/mL. For the IS, a solution of 1 mg/mL was prepared in methanol. These solutions were divided into aliquots and stored at −80 °C. Calibration standards and quality control (QC) samples were prepared by spiking the blank plasma samples with known concentrations of standard solutions of the drugs.

2.3. Chromatographic and mass spectrometric conditions

The UPLC-MS/MS analysis was performed on an Acquity UPLC H-Class system coupled to the XEVO TQD tandem mass spectrometry module (Waters, Milford, MA, USA) with an Acquity UPLC BEH C18 column (2.1 mm × 50 mm; 1.7 µm,) and an Acquity UPLC BEH C18 VanGuard Precolumn (2.1 mm × 5 mm; 1.7 µm). The column temperature was set at 40 °C, and the autosampler was kept at 10 °C.

The mobile phase was composed of 60% of ammonium acetate (5 mM) with 0.1% formic acid and 40% of acetonitrile. Isocratic elution mode was used with a flow rate of 0.4 mL/min. The injection volume and total run time were 20 µL and 5 min, respectively.

The operating parameters were positive electrospray ionization mode, capillary voltage 0.80 kV; source temperature 150 °C; desolvation temperature 500 °C; desolvation gas flow 1000 L/h (N_2, 99.9% purity). High purity argon was used as collision gas and multiple reaction monitoring (MRM) was applied for detection of the analytes. The specific mass spectrometry conditions and transitions used for quantification and identification of the drugs and IS are shown in Table 1. The MassLynx v.4.2 software with the TargetLynx XS Application Manager (Waters) was used to acquire and process all data.

2.4. Sample preparation

Plasma samples were prepared as follows: 400 µL of acetonitrile containing 100 ng/mL of IS were added to 200 µL of plasma. The

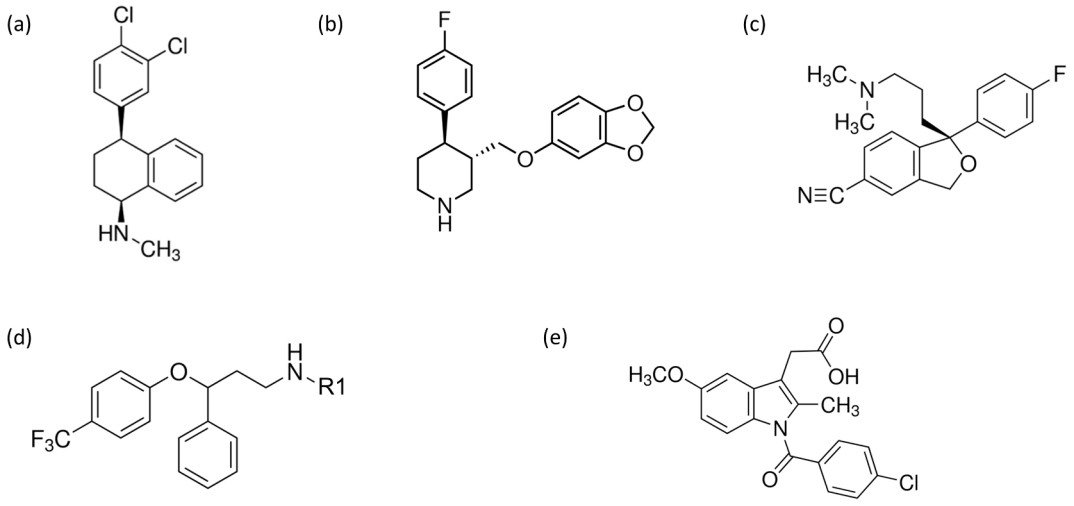

Fig. 1. Chemical structures of the analytes. (a) sertraline, (b) paroxetine, (c) escitalopram, (d) fluoxetine: R1 = CH3, norfluoxetine: R1 = H, (e) indomethacin (internal standard).

Table 1
Mass spectrometry conditions.

Analyte	Transition (*m/z*)	Cone voltage (V)	Collision energy (V)	RT (min)
SRT	308.1 > 160.90[a]	20	24	1.10
	308.1 > 277	20	10	
PRX	330.15 > 70.05[a]	52	28	0.73
	330.15 > 192.1	52	21	
ESC	325.2 > 109.05[a]	40	23	0.63
	325.2 > 262.11	40	18	
FLX	310.16 > 43.9[a]	24	10	1.03
	310.16 > 148.02	24	8	
NFLX	296.16 > 29.9[a]	18	7	0.93
	296.16 > 134.0	18	4	
IND (IS)	558.08 > 138.08[a]	34	18	3.56

SRT: sertraline; PRX: paroxetine; ESC: escitalopram; FLX: fluoxetine; NFLX: norfluoxetine; IND: indomethacin (internal standard); [a]: transition for quantification; RT: retention time

mixture was vortexed 30 s and then centrifuged at 20,817 × g for 20 min at 4 °C. The supernatant (450 µL) was evaporated to dryness in a vacuum concentrator (Eppendorf, Concentrator Plus) at 60 °C for 110 min. Then, the residue was reconstituted with 100 µL of mobile phase, vortexed 30 s, and centrifuged at 20,817 × g for 10 min at 4 °C. Finally, supernatant was transferred to glass vials and 20 µL were injected into UPLC system for analysis.

2.5. Method validation

The analytical method was fully validated using drug free plasma, according to the Food and Drug Administration (US FDA) bioanalytical method validation guidance [27].

2.5.1. Selectivity

Selectivity was evaluated by analyzing drug free plasma samples from six different donors. To consider a sample without interfering compounds, the response of these compounds should be less than 20% of the lower limit of quantification (LLOQ) for the analytes and less than 5% for IS.

2.5.2. Linearity, accuracy, and precision

Linearity was assessed by at least three calibration curves with at least six concentration levels, considering the therapeutic range of the analyzed drugs. A blank plasma sample (matrix processed without analyte and IS) and a zero sample (matrix processed with IS) were included in each calibration curve evaluated. The calculated concentration of the calibration standards should be ± 15% of the nominal concentration and ± 20% for the LLOQ nominal concentration.

Precision and accuracy were estimated by assays performed on the same day (intra-assay) and three assays performed on at least two different days (inter-assay) of the LLOQ and QC samples at low (LQC), medium, and high (HQC) concentration. The assay was repeated five times per run.

The mean concentration obtained from the QC samples should be ± 15% of the nominal concentration and ± 20% of the nominal concentration for the LLOQ to accept accuracy. The precision was determined by the coefficient of variation (CV) which should be less than 15% for QC samples and should be less than 20% for LLOQ.

2.5.3. Carryover and matrix effect

Carryover was determined with the upper limit of quantification (ULOQ) and a blank plasma sample. The assay was performed by one injection of the blank plasma sample followed by one injection of the ULOQ and then two more injections of the blank plasma sample. Analytical response of the later should not exceed 20% of LLOQ and 5% of average IS analytical response.

The matrix factor (MF) for each drug and IS was evaluated with the LQC and HQC in six different lots of matrix. The MF was calculated by dividing the response of blank matrix spiked with analyte after extraction and the response of analyte in the absence of matrix (mobile phase). Also, the IS-normalized MF (IS-NMF) was calculated by dividing the MF of the analyte and MF of the IS. The CV of the IS-NMF should be less than 15% to be accepted.

2.5.4. Stability

The stability of the drugs in the matrix was tested by quintuplicate analysis of LQC and HQC at different temperature conditions: room temperature, autosampler, storage temperature and after freeze–thaw cycles. Samples were stable if the mean concentration obtained at each level was within ± 15% of the nominal concentration.

2.6. Analysis of patient samples

Plasma samples were obtained from patients of the Psychiatry and Psychology service of CHIMP of San Luis Potosí, México.

The study protocol was approved by the Research and Ethics Committee of CHIMP (registration number 46–17) and Ethics in Research and Teaching Committee of the Faculty of Chemical Sciences of the Autonomous University of San Luis Potosi, Mexico (registration number CEID2018-065). The study was carried out according to the Declaration of Helsinki and all patients included provided written informed consent.

Outpatients older than 18 years, with a diagnosis of psychiatric disorder according to International Classification of Diseases 10 (ICD-10) who received treatment with any of the SSRI studied were included. Pregnant women, patients on concomitant treatment with another antidepressant, and patients with no clinical information available were not included.

One blood sample per patient was obtained preferably prior to the next dose and once steady state drug concentration was achieved (at least 15 days after initiation of treatment with SRT, PRX, ESC, and at least 30 days for FLX).

Blood samples were collected in EDTA Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) centrifuged at 304 × g for 20 min and the plasma was recovered and stored at −80 °C until analysis.

At the time of blood sampling, information regarding the duration of SSRI treatment, time of the last dose, concomitant medication, possible adverse effects, smoking, and alcohol habits were retrieved. Anthropometric information and diagnosis were obtained from the medical record.

3. Results

3.1. Analytical method validation

3.1.1. Selectivity

Chromatograms of the studied drugs are shown in Fig. 2. No interferences at the retention time of the drugs and IS were detected for endogenous and exogenous components in plasma.

3.1.2. Linearity, accuracy, and precision

Calibration curves were performed with eight levels of standard concentration for each drug (5, 10, 25, 50, 100, 200, 400, and 800 ng/mL) and a simple linear least squares regression was applied to determine linearity.

The method was linear at a range of 5 to 800 ng/mL for the evaluated drugs with determination coefficients>0.9911 and recovery values ranged from 92% to 106%. The calibration standards and the LLOQ (which was defined as the lowest concentration on the calibration curve) showed CV less than 7%.

The accuracy was ranged from 91% to 112% (intra-assay) and from 89% to 112% (inter-assay) whereas precision ranged CV values from 3.1% to 14.88% (intra-assay) and from 3.60% to 14.74% (inter-assay).

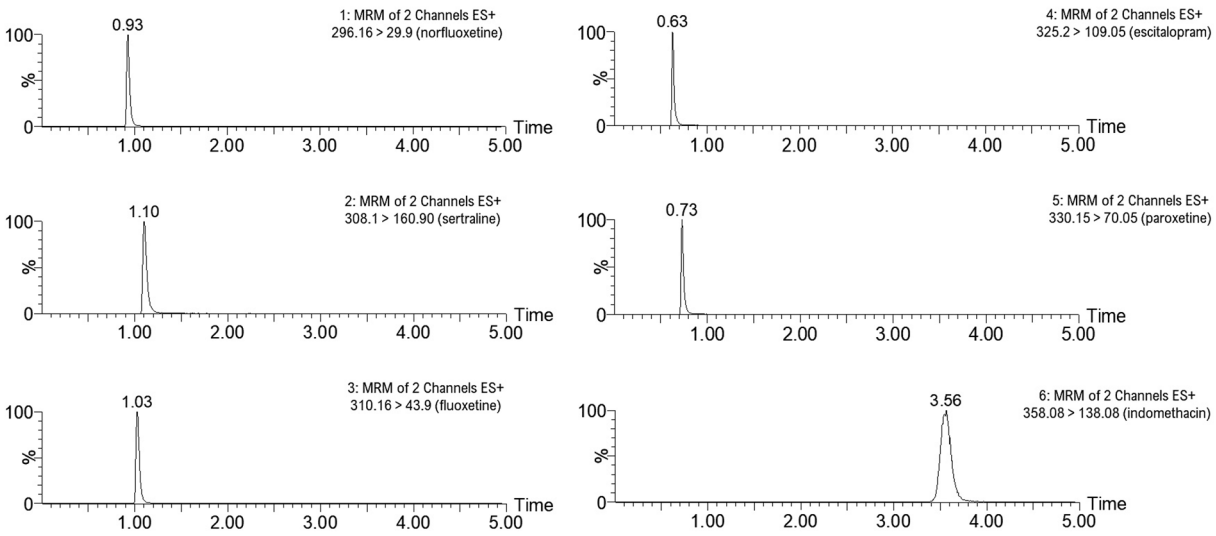

Fig. 2. Representative chromatograms of norfluoxetine, sertraline, fluoxetine, escitalopram, paroxetine, and indomethacin (internal standard) with retention time and mass transition (*m/z*).

The accuracy and precision results for LLOQ and for each QC sample are summarized in Table 2.

3.1.3. Carryover and matrix effect

The carryover effect was less than 1.16% for all drugs and 0.70% for IS. The estimation of IS-NMF in the six matrix lots ranged CV values from 5.67% to 12.24%, the results for each drug are shown in Table 2.

3.1.4. Stability

The studied drugs were stable at room temperature (18 °C) for 4 h, in the autosampler (10 °C) for 24 h, at storage temperature (-80 °C) for 30 days and after three freeze–thaw cycles. For all conditions, mean concentrations were within ± 15% of the nominal concentration. The results of the conditions tested are summarized in Table 3.

3.2. Analysis of patient samples

The validated method was applied for detection and simultaneous quantification of SSRI in 73 plasma samples from outpatients with psychiatric disorders.

The age of the included patients ranged from 18 to 83 years with a mean of weight, height and body mass index of 70.80 kg, 1.62 m and 27.02 kg/m^2, respectively. Characteristics of the included patients, comedication and the quantified concentrations for each analyzed drug are presented in Table 4.

Major depressive disorder (45.2%), generalized anxiety disorder (27.4%), mental and behavioral disorders due to psychoactive substance use (6.9%), social phobia (5.48%), schizophrenia (4.11%), mixed anxiety-depressive disorder (4.11%) and dysthymia (4.11%) were the most frequent diagnosis. These psychiatric disorders were mainly

Table 2
Linearity, matrix effect, accuracy and precision of UPLC MS-MS method.

Drug	R^2	Nominal concentration (ng/mL)	IS-NMF (%CV)(n = 3)	Intra-assay (n = 5)			Inter-assay (n = 15)		
				Obtained concentration mean ± SD (ng/mL)	Precision(% CV)	Accuracy (%)	Obtained concentration mean ± SD (ng/mL)	Precision(% CV)	Accuracy (%)
SRT	0.991	5.0		5.28 ± 0.53	10.04	105.60	5.20 ± 0.52	10.00	104.00
		15.0	7.89	15.16 ± 0.47	3.10	101.07	15.12 ± 0.75	4.96	100.80
		80.0		78.76 ± 6.06	7.69	98.45	75.31 ± 8.37	11.11	94.13
		600.0	11.83	600.91 ± 64.22	10.69	100.15	582.19 ± 56.91	9.78	97.03
PRX	0.995	5.0		5.10 ± 0.61	11.96	102.00	5.04 ± 0.49	9.72	100.80
		15.0	6.06	13.98 ± 1.81	12.95	93.20	13.64 ± 1.78	13.05	90.93
		80.0		81.48 ± 9.23	11.33	101.85	80.36 ± 8.01	9.97	100.45
		600.0	5.67	590.02 ± 83.99	14.24	98.34	589.37 ± 54.74	9.29	98.23
ESC	0.992	5.0		5.12 ± 0.63	12.30	102.40	4.90 ± 0.58	11.84	98.00
		15.0	10.72	13.66 ± 1.03	7.54	91.07	14.60 ± 2.00	13.70	97.33
		80.0		89.61 ± 7.38	8.24	112.01	82.76 ± 12.20	14.74	103.45
		600.0	8.46	613.69 ± 67.38	10.98	102.28	612.55 ± 76.35	12.46	102.09
FLX	0.996	5.0		5.28 ± 0.75	14.20	105.60	5.30 ± 0.67	12.64	106.00
		15.0	10.38	13.90 ± 1.16	8.35	92.67	13.89 ± 1.88	13.53	92.60
		80.0		84.80 ± 4.98	5.87	106.00	81.33 ± 7.63	9.38	101.66
		600.0	11.06	571.04 ± 45.76	8.01	95.17	538.00 ± 48.14	8.95	89.66
NFLX	0.995	5.0		5.42 ± 0.26	4.80	108.40	5.60 ± 0.28	5.00	112.00
		15.0	12.24	14.18 ± 2.11	14.88	94.53	14.66 ± 1.80	12.28	97.73
		80.0		76.62 ± 4.61	6.02	95.78	84.08 ± 5.27	6.27	105.1
		600.0	10.92	619.06 ± 21.40	3.46	103.18	547.38 ± 19.69	3.60	91.23
IND (IS)	–	100.0	–	100.07 ± 4.68	4.67	100.07	99.72 ± 4.31	4.32	99.72

SRT: sertraline; PRX: paroxetine; ESC: escitalopram; FLX: fluoxetine; NFLX: norfluoxetine; IND: indomethacin (internal standard); R^2: determination coefficient; IS-NMF: Internal Standard-Normalized Matrix Factor; SD: standard deviation; CV: coefficient of variation

Table 3
Stability results at different conditions (n = 5).

Drug	Nominal concentration (ng/mL)	Processed sample 18 °C, 4 h		Autosampler 10 °C, 24 h		Freeze-thaw cycles		Storage −80 °C, 30 days	
		Mean (ng/mL)	RE (%)	Mean (ng/mL)	RE (%)	Mean (ng/mL)	RE (%)	Mean (ng/mL)	RE (%)
SRT	15.0	15.56	3.73	14.58	−2.80	15.80	5.33	15.03	0.22
	600.0	616.51	2.75	638.64	6.44	634.90	5.82	564.83	−5.86
PRX	15.0	15.32	2.13	14.09	−6.07	12.95	−13.67	15.13	0.89
	600.0	587.96	−2.01	546.53	−8.91	567.93	−5.35	545.53	−9.08
ESC	15.0	14.85	−1.00	13.34	−11.07	12.92	−13.87	14.37	−4.22
	600.0	640.99	6.83	612.64	2.11	557.50	−7.08	634.21	5.70
FLX	15.0	14.77	−1.53	14.07	−6.20	13.57	−9.53	14.87	−0.89
	600.0	569.80	−5.03	567.37	−5.44	538.37	−10.27	559.90	−6.68
NFLX	15.0	15.57	3.80	14.13	−5.80	13.65	−9.00	15.70	4.67
	600.0	559.67	−6.72	555.27	−7.46	557.93	−7.01	591.47	−1.42

SRT: sertraline; PRX: paroxetine; ESC: escitalopram; FLX: fluoxetine; NFLX: norfluoxetine; RE: relative error

Table 4
Patient characteristics (n = 73).

Variable	Value
Sex (n) (female/male)	47/26
Age (years)	45 (18 – 83)
Weight (kg)	69.89 (42.00 – 115.00)
Height (m)	1.62 (1.45 – 1.84)
Body mass index (kg/m^2)	27.24 (16.38 – 42.76)
SSRI concentration (ng/mL)	FLX (n = 22): 78.85 (13.70 – 310.20)
	NFLX (n = 22): 71.30 (10.70 – 185.40)
	ESC (n = 18): 35.60 (11.10 – 68.60)
	SRT (n = 17): 37.90 (3.00 – 158.30)
	PRX (n = 16): 62.40 (8.50 – 124.00)
Smokers (n)	8 (11.0%)
Alcohol (n)	11 (15.1%)
Comedication	
Metformin (n)	12 (16.4%)
Clonazepam (n)	11 (15.1%)
Enalapril (n)	9 (12.3%)
Folic acid (n)	9 (12.3%)
Olanzapine (n)	9 (12.3%)
Risperidone (n)	9 (12.3%)
Levothyroxine (n)	8 (11.0%)
Pregabalin (n)	7 (9.6%)
Quetiapine (n)	7 (9.6%)

Continuous data are presented as median (range); categorical data are presented as frequency (%); SRT: sertraline; PRX: paroxetine; ESC: escitalopram; FLX: fluoxetine; NFLX: norfluoxetine

treated with FLX (n = 22) with doses ranged from 20 to 40 mg/day, followed by ESC (n = 18) with doses from 5 to 20 mg/day, SRT (n = 17) with doses from 25 to 100 mg/day and PRX (n = 16) with doses from 20 to 40 mg/day, respectively.

Of the total samples analyzed, 18 patients (24.6%) showed lower concentrations whereas 9 patients (12.3%) showed concentrations above the specific therapeutic range of each SSRI. The results of the concentrations obtained in the patient samples are shown in Fig. 3.

4. Discussion

In the present report, an analytical method by UPLC-MS/MS was developed and validated for the simultaneous quantification of SRT, ESC, PRX, FLX and its main metabolite NFLX in a considerably short run time (5 min).

The main factor that allowed to obtain this run time was the use of isocratic elution mode. In this elution, all components of the mobile phase are mixed and pumped together as a single eluent [28], which causes a higher separation speed because re-equilibration of the column is omitted; as a general rule, the column must be washed with at least 10 volumes of initial eluent before the next run can be continued [29,30].

The run time obtained is equal to or shorter than those reported in simultaneous LC-MS/MS methods for the quantification of antidepressant and antipsychotic drugs, in which gradient elution is used for chromatographic separation and have run times between 5 and 30 min; moreover, the necessary time to re-equilibrate the column between one sample and another ranged from 1.9 to 6 min [16–21,23,24].

For sample processing, a simple PPT method was chosen using acetonitrile as precipitating agent at 2:1 proportion that previously was used for the simultaneous analysis of antidepressants and antipsychotics drugs [24]. However, the addition of IS in acetonitrile simplified the extraction method and reduced the number of pipetting steps.

The recovery results obtained for drugs (92–106%) demonstrate that this procedure is comparable with those used in simultaneous UPLC-MS/MS methods in which PPT was used with precipitating agents such as methanol with 0.1% formic acid (recovery 91–105%) [23], 0.05% zinc sulfate in acetonitrile/methanol (recovery 87.1–110%) [20] and methanol (recovery 92–111%) [16].

The chromatographic conditions used provide adequate separation, peaks with acceptable symmetry and adequate sensitivity for drug quantification. It has been reported that acidic eluents with ammonium formate, ammonium acetate and acetic acid are often used for SSRI bioanalysis [29]. The addition of formic acid to the mobile phase is related to a decreased peak tails and fronts, as well as improved sensitivity because it promotes ionization of the analytes [31,32].

The range of concentrations studied (5–800 ng/mL) was based on the therapeutic and alert ranges proposed by the AGNP-TDM guidelines for SSRI [10]. Some methods have proposed lower LLOQ for SSRI than the one presented [18,21,23,24], however, for the purposes of this study, the range studied is sufficient and adequate to perform TDM of SSRI.

For the TDM of FLX and its metabolite NFLX, both must be quantified to determine whether a concentration is within the therapeutic range [10]. The current method was able to simultaneously determine these two analytes, which is an advantage over methods in which NFLX quantification is omitted [21,23,24].

Simultaneous quantification of CIT and ESC enantiomers could not be performed with the chromatographic and mass spectrometric conditions established in the method because both compounds have identical chromatographic and spectrometric behavior with current conditions since an achiral column has been used. However, for the purposes of this study it did not represent a drawback because in clinical practice both drugs are not prescribed simultaneously and the drug that the patient is taking is known.

For the simultaneous analysis of SSRIs, several drugs have been proposed as IS, such as clomipramine [13], protriptyline [14], melperone [12], fluvoxamine [15], glibenclamide [23], and alprenolol [20]. IND was chosen as IS in the present study, which was a reasonable choice because it is a nonsteroidal anti-inflammatory drug used to relieve or reduce pain that is rarely prescribed in this study patients, therefore the potential interference with this analytical method is minimal.

On the other hand, although IND has little structural similarity, it

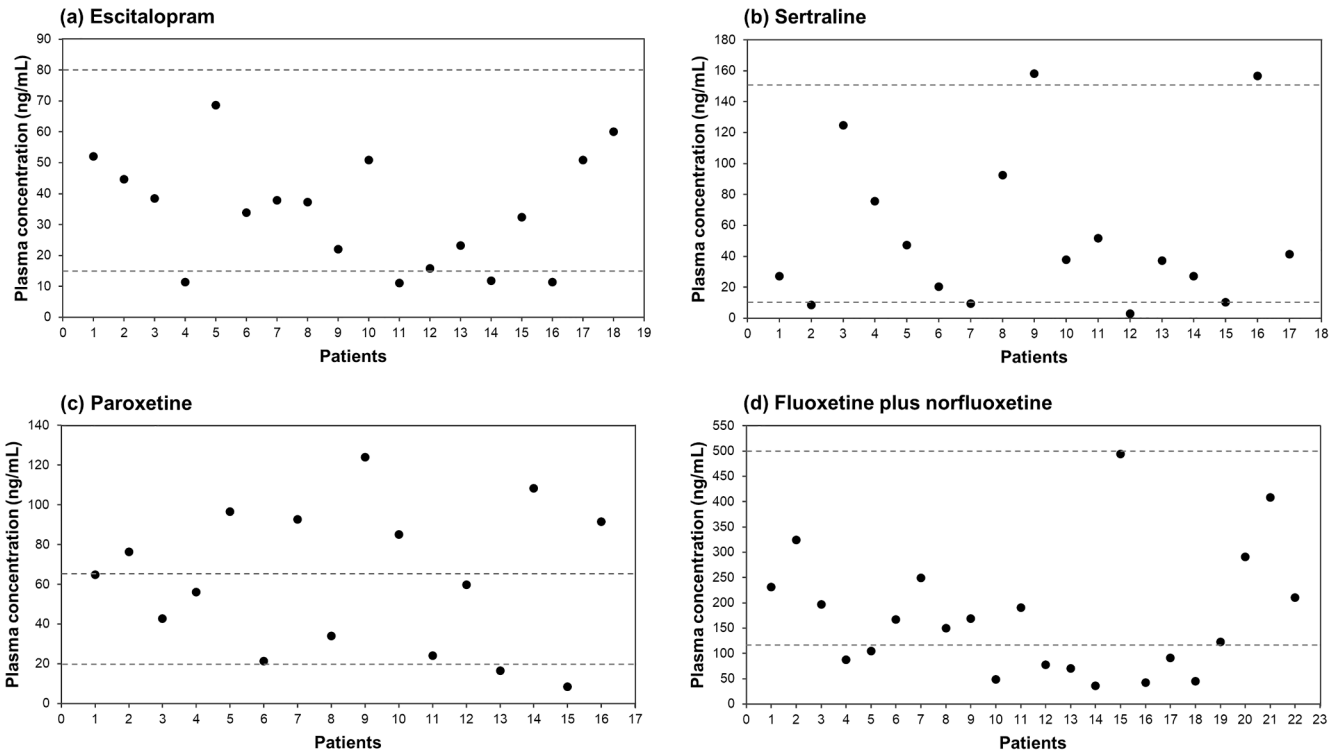

Fig. 3. Concentrations of the drugs studied in the patients' plasma samples. The therapeutic reference ranges for each drug are shown in dashed line.

exhibited similar chromatographic behavior to SSRI, which allowed its elution and separation of the analytes. Furthermore, IND is an acceptable IS because no ion supression/enhancernent was observed in its use, nor did it affect the sensitivity or recovery of the analytes.

Regarding the analysis of patient samples, the most frequent psychiatric disorders were depression and anxiety. The 36.9% of the patient included had concentrations outside therapeutic ranges. Compliance rates are often low in the treatment of mental disorders such as depression [9].

Subtherapeutic concentrations may reflect poor compliance, drug-drug interactions, or the presence of an ultrafast metabolizing genotype [9]. On the other hand, at supratherapeutic concentration there is a higher risk of adverse effects, intolerance, toxic effects, and may even reflect the presence of a poor metabolizing genotype, therefore a dose adjustment may be necessary [9,10]. In the patients studied, there were no drug-drug interactions that could affect the SSRI concentrations achieved.

Based on the results obtained, the development and validation of cost-effective analytical methods that allow the quantification of SSRIs with adequate values of accuracy and precision could contribute to increase compliance rates, reveal non-responders and finally to perform the necessary dose adjustments to individualize therapy with these drugs.

5. Conclusion

A simultaneous method for the quantification of the main SSRI prescribed in clinical practice was developed and validated. A simple PPT extraction method was used, and the drugs were eluted in an isocratic mode. The method was validated in accordance with the FDA guidelines for selectivity, linearity, precision, accuracy, and stability. Current results probe that this method can be applied for routine analysis in clinical practice, because it has a wide range of concentrations, a simple sample preparation and a short run time, which consequently reduces the costs associated to TDM purposes.

CRediT authorship contribution statement

Cinthya Eloisa Chávez-Castillo: Investigation, Writing – original draft, Writing – review & editing. **Julia Sagahón-Azúa:** Investigation, Writing – original draft, Writing – review & editing. **Karla Itzel Velasco-Gloria:** Investigation, Writing – original draft. **Susanna Edith Medellín-Garibay:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Rosa del Carmen Milán-Segovia:** Methodology, Resources, Visualization. **Silvia Romano-Moreno:** Conceptualization, Methodology, Resources, Writing – review & editing, Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Anexo II.

Population pharmacokinetics of sertraline in psychiatric and substance use disorders

Autores:

Cinthya Eloisa Chávez-Castillo, Susanna Edith Medellín-Garibay, Rosa del Carmen Milán-Segovia, Sergio Zarazúa-Guzmán, Helgi Jung-Cook, Marisol Orocio-Contreras, Silvia Romano-Moreno

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Authors
Chávez-Castillo, Cinthya Eloisa
Medellín-Garibay, Susanna
Milán-Segovia, Rosa del Carmen
Zarazúa-Guzmán, Sergio
Jung-Cook, Helgi
Orocio-Contreras, Marisol
Romano-Moreno, Silvia

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Keywords:	Population pharmacokinetics, Sertraline, Dual disorders, Dual pathology, CYP2D6, CYP2C19
Generic Drug Names:	Sertraline
Abstract:	The aim of this study was to characterize the population pharmacokinetics of sertraline in Mexican patients with psychiatric and substance use disorders. Fifty-nine patients (13 to 76 years old) treated with doses of sertraline between 12.5 - 100 mg/day were included. Plasma sertraline concentrations were determined in blood samples and five of the main substances of abuse were determined by rapid tests in urine samples. Demographic, clinical and pharmacogenetic factors were also evaluated. Population pharmacokinetic analysis was performed using NONMEM® software with first order conditional estimation method. A one-compartment model with proportional residual error adequately described the sertraline concentrations versus time. CYP2D6*2 polymorphism and CYP2C19 phenotypes significantly influenced sertraline clearance which had a population mean value of 66 L/h in the final model. The absorption constant and volume of distribution were fixed at 0.855 1/h and 20.2 L/kg, respectively. The model explained 11.3% of the interindividual variability in sertraline clearance. The presence of the CYP2D6*2 polymorphism caused a 23.1% decrease in sertraline clearance, whereas patients with intermediate and poor phenotype of CYP2C19 showed 19.06% and 48.26% decreases in sertraline clearance, respectively. The model was internally validated by bootstrap and visual predictive check. Finally, stochastic simulations were performed to propose dosing regimens to achieve therapeutic

	levels that contribute to improve treatment response.

Title

Population pharmacokinetics of sertraline in psychiatric and substance use disorders

Authors

Cinthya Eloisa Chávez Castillo, MSc[1], Susanna Edith Medellín Garibay, PhD[1], Rosa del Carmen Milán Segovia, PhD[1], Sergio Zarazúa Guzmán, PhD[1], Helgi Jung Cook, PhD[2,3], Marisol Orocio Contreras, MD[4], Silvia Romano Moreno, PhD[1]

Affiliations

[1]Pharmacy Department, Faculty of Chemical Sciences, Autonomous University of San Luis Potosi, Mexico.
[2]Pharmacy Department, Faculty of Chemistry, National Autonomous University of Mexico, Mexico.
[3]Neuropsychopharmacology Department, National Institute of Neurology and Neurosurgery, Mexico.
[4]Centros de Integración Juvenil, A.C., San Luis Potosi, Mexico.

Corresponding author:

Silvia Romano Moreno, PhD.
6 Dr. Manuel Nava Avenue, 78210 San Luis Potosi, San Luis Potosi, Mexico.
Email: srm@uaslp.mx.

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Data sharing

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Conflicting Interests

The authors declare no conflicts of interest

Abstract

The aim of this study was to characterize the population pharmacokinetics of sertraline in Mexican patients with psychiatric and substance use disorders. Fifty-nine patients (13 to 76 years old) treated with doses of sertraline between 12.5 - 100 mg/day were included. Plasma sertraline concentrations were determined in blood samples and five of the main substances of abuse were determined by rapid tests in urine samples. Demographic, clinical and pharmacogenetic factors were also evaluated. Population pharmacokinetic analysis was performed using NONMEM® software with first order conditional estimation method. A one-compartment model with proportional residual error adequately described the sertraline concentrations versus time. CYP2D6*2 polymorphism and CYP2C19 phenotypes significantly influenced sertraline clearance which had a population mean value of 66 L/h in the final model. The absorption constant and volume of distribution were fixed at 0.855 1/h and 20.2 L/kg, respectively. The model explained 11.3% of the interindividual variability in sertraline clearance. The presence of the CYP2D6*2 polymorphism caused a 23.1% decrease in sertraline clearance, whereas patients with intermediate and poor phenotype of CYP2C19 showed 19.06% and 48.26% decreases in sertraline clearance, respectively. The model was internally validated by bootstrap and visual predictive check. Finally, stochastic simulations were performed to propose dosing regimens to achieve therapeutic levels that contribute to improve treatment response.

Keywords

Population pharmacokinetics, sertraline, dual disorders, dual pathology, CYP2D6, CYP2C19

Introduction

Dual pathology is a term used to refer to a dual diagnosis disorder, in which a substance use disorder and other psychiatric disorder coexist.[1]

Dual pathology has multiple diagnostic and therapeutic difficulties such as it is complicated to differentiate between the symptomatology of mental illness from disorders associated with substance abuse, noncompliance during treatment and rehabilitation, higher rates of relapse and rehospitalization, as well as greater psychosocial impairment due to legal problems, violence, homelessness, lack of community support, suicidal ideation and increased risk of sexually transmitted diseases, which increases health care costs.[2,3]

Pharmacological treatment of dual pathology is considered supportive rather than curative and is intended to relieve distressing symptoms, help reduce craving for substance use, and potentially contribute to abstinence.[4]

Selective serotonin reuptake inhibitors have become one of the first lines of pharmacological treatment of dual pathology,[4] within this group is sertraline that is an antidepressant approved for the treatment of depression, anxiety disorder, panic disorder (with or without agoraphobia disorder), post-traumatic stress disorder and obsessive-compulsive disorder.[5] Additionally, this drug is widely used because it is unlikely to have abuse potential[6] and has a relatively favorable safety profile in cases of overdose.[7,8]

The pharmacokinetics of sertraline is dose proportional in the range of 50 to 200 mg reaching peak plasma concentrations at 4-8 h after oral administration and approximately 98% of the circulating drug is bound to plasma proteins.[5,9,10] It has extensive hepatic metabolism by multiple CYP-450 isoenzymes such as CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, which generate a weakly active metabolite, N-desmethylsertraline,

that does not contribute to clinical effect.[11–13] The half-life of sertraline is approximately 26 h, thus steady state is reached at 7 days of daily dosing.[5,9,14]

Several studies have identified that factors such as age,[14,15] gender,[14] hepatic function,[16] serum concentrations of N-desmethylsertraline,[17] metamizole comedication,[18] grapefruit juice[19] and polymorphisms CYP2C19[20–22] and CYP2B6[23,24] could influence sertraline pharmacokinetics, leading to high interindividual variability. However, there are no established dosing regimens based on these covariates that could be applied in clinical practice.

Therefore, the objective of this study was to develop and validate a population pharmacokinetic model of sertraline to determine the covariates that explain the interindividual and intraindividual variability of pharmacokinetic parameters in patients with dual pathology in order to propose dosing regimens that could contribute to improved treatment outcomes in these patients.

Methods

The study was conducted at Centros de Integración Juvenil (CIJ) of San Luis Potosi, Mexico. The study protocol was approved by the Scientific Research Committee of CIJ (registration number 203-19) and by the Research and Teaching Ethics Committee of the Faculty of Chemical Sciences of the Autonomous University of San Luis Potosi, Mexico (registration number CEID2018-065). The study was conducted in accordance with the Declaration of Helsinki. Each patient received detailed information about the research protocol, after which written informed consent was obtained, and in the case of patients under 18 years of age, additionally the parents or tutor signed a written informed consent for samples collection and subsequent analysis.

Subjects

Patients were examined by the therapists and the psychiatrist of CIJ. Diagnosis of psychiatric disorders and substance use disorders were determined by the Mini-International Neuropsychiatric Interview (MINI-Plus) which is a brief structured diagnostic interview that explores the major psychiatric disorders of axis I of the Diagnostic and Statistical Manual of Mental Disorders-IV and International Classification of Diseases-10. Outpatients older than 12 years, with a diagnosis of psychiatric disorder and substance use disorder who were treated with sertraline were included. Pregnant women, patients concomitantly treated with another antidepressant, patients with no clinical information available or those suspected of therapeutic noncompliance were not included.

Blood and urine sampling

Once treatment with sertraline was initiated, a blood sample and a urine sample were obtained at least 7 days after initiation of treatment. At another subsequent visit, a second blood and urine sample were obtained at least 30 days after the first sample.

Blood samples were collected in 4 mL capacity EDTA Vacutainer® tubes, the samples were then centrifuged at 1300 rpm for 20 min and the plasma was stored at -80 °C until analysis. The collected urine samples were used to perform a multidrug rapid test (Instant View®) for marijuana, amphetamines, methamphetamines, cocaine and benzodiazepines.

In each of the samplings, an interview was conducted with the patient to obtain information about the treatment with sertraline, substance use, and the Beck's Anxiety Inventory and Beck's Depression Inventory were also applied. Information on anthropometric variables, diagnosis, substances consumed, duration of treatment and biochemical parameters such as

glucose, total cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, serum creatinine, hemoglobin, hematocrit and platelets were obtained from each patient's clinical record.

Bioanalytical method

Plasma concentrations of sertraline were determined by a validated ultra-high performance liquid chromatography (UPLC MS-MS) method with the conditions described in a previous study.[25] Briefly, to 200 µL of plasma, 400 µL of acetonitrile containing 100 ng/mL of indomethacin as internal standard were added. The mixture was centrifuged and a 450 µL aliquot was evaporated to dryness. Then, the residue was reconstituted with 100 µL of mobile phase consisting of 60% ammonium acetate (5 mM) with 0.1% formic acid and 40% acetonitrile. The analysis of plasma samples was performed on an Acquity UPLC H-Class system coupled to the XEVO TQD tandem mass spectrometry module (Waters, Milford, MA, USA) with an Acquity UPLC BEH C18 column (2.1 mm × 50 mm; 1.7 µm) at 40 °C and an Acquity UPLC BEH C18 VanGuard Precolumn (2.1 mm × 5 mm; 1.7 µm). An isocratic elution mode was used at a flow rate of 0.4 mL/min, an injection volume of 20 µL and a run time of 5 min, respectively.

The method was linear in a range of 5 to 800 ng/ mL, the accuracy for sertraline was ranged from 98.5% to 105.6% for intra-assay and from 94.1% to 104.0% for inter-assay. The variation coefficients ranged from 3.1% to 10.7% for intra-assay and from 5.0% to 11.1% for inter-assay precision.

Genotyping

Genomic DNA was obtained from a blood sample from the patient using the commercial Blood DNA Preparation kit® from Jena Bioscience to determine single nucleotide polymorphisms by real-time PCR with and with the use of TaqMan® probes. CYP2D6*2 (rs16947), CYP2D6*4 (rs3892097), CYP2D6*10 (rs1065852), CYP2D6*17 (rs28371706), CYP2D6*41 (rs28371725), CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910), CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560) and CYP2B6*9 (rs3745274) were determined on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA).

Population pharmacokinetic analysis

The concentrations of sertraline versus time data were analyzed by nonlinear mixed effects modelling approach using NONMEM® software v.7.4 (Icon Development Solutions, Dublin, Ireland). For the model development and graphical presentation, Pearl-speaks.NONMEM® (PsN) (v.5.3.0; https://uupharmacometrics.github.io/PsN/), Xpose (v.0.4.13; https://uupharmacometrics.github.io/xpose/) R® (v.4.1.2; https://www.r-project.org/) and Pirana® v.2.9.9 (Certara USA, Inc., NJ, USA) were used as auxiliary software tools.

One and two compartment models, with first order oral absorption and elimination kinetics were assessed and compared to determine the best structural model. Proportional and exponential error models were tested to determinate interindividual variability (IIV). For the residual unexplained variability (RV), additive, proportional and combined (additive + proportional) error models were evaluated with the First Order Conditional Estimation (FOCE) to calculate the mean for the interindividual and residual variability of the parameters.

The initial estimates of absorption rate constant (Ka), clearance (CL) and volume of distribution (V) were taken from previous pharmacokinetic studies of sertraline.[14,26–28]. Model evaluation was based on a likelihood ratio test using the objective function value (OFV).[29] A base model was initially developed, and the covariate model was performed by the stepwise covariate modelling technique for preliminary selection.

Continuous covariates such age, weight, height, serum glucose, urea, triglycerides, total cholesterol, creatinine, Crockroft-Gault estimate of creatinine clearance, albumin, aspartate aminotransferase, alanine aminotransferase, hemoglobin, hematocrit, platelets, body mass index, Beck's Anxiety Inventory scores and Beck's Depression Inventory scores were introduced into the population model using linear, allometric or exponential functions.

The categorical covariates evaluated were sex, tobacco, alcohol and grapefruit juice consumption, comorbidities, comedication, genotypes, phenotypes and urine multidrug rapid test results. Discrete covariates such as sex was coded as 0 for female or 1 for male; tobacco, alcohol and grapefruit juice consumption were coded as 0 for absent or 1 for present in the last week before sampling; comorbidities, comedication and urine multidrug rapid test results were coded as 0 for absent or 1 for present; genotypes were coded as 0 for homozygous for reference allele, 1 for heterozygous or homozygous for mutated allele; phenotypes were coded as 0 for poor metabolizer (PM), 1 for intermediate metabolizer (IM), 2 for extensive metabolizer (EM) and 3 for rapid metabolizer (RM).

Missing data from the continuous covariates were substituted with the median covariate value (covariates with >20% of missing values were discarded); there were no missing data in the categorical covariates.

In forward addition step, the criterion for estimating statistical significance was that the reduction in OFV should be at least 3.84 ($\chi^2 = 3.84$, $p < 0.05$). Covariates were added one

by one into the model until no further addition of covariates was significant. The full model obtained was subjected to backward elimination, a more stringent criterion for statistical significance was used ($\chi^2 = 6.63$, $p < 0.01$) to determine the final model. Additional criteria used in evaluating alternative models was the minimization of interindividual variability, the percentage relative standard error and the improvement in the visual inspection of the diagnostic plots.

The accuracy of the final model was evaluated by 1000 runs of the bootstrap procedure to calculate the median and nonparametric 95% confidence intervals (95% CI) of parameters. Internal predictive ability was evaluated by visual predictive check (VPC). Finally, 200 stochastic simulations of trough sertraline concentrations based on the final population model were performed to propose dosing regimens.

Results

Demographic and clinical data

The study population consisted of 59 patients with DP treated with sertraline, from which a total of 75 observations of plasma concentrations were obtained and used for the development of the population pharmacokinetic analysis.

20.3% of the patients were under 18 years of age and 79.7% were adults, most of the patients were male (55.9%). Demographic and clinical characteristics of the participants are presented in Table 1.

The most common substance use disorder were alcohol abuse or dependence (69.5%), nicotine dependence (32.2%), amphetamine abuse or dependence (27.1%), marijuana abuse or dependence (22.0%), inhalant abuse (5.1%) and cocaine dependence (6.8%).

The other psychiatric disorder presented by patients were major depressive disorder (37.3%), anxiety disorder (33.9%), amphetamine-induced mood and anxiety disorder (25.4%), borderline personality disorder (20.3%), alcohol-induced mood and anxiety disorder (16.9%), persistent depressive disorder (15.3%), dependence personality disorder (8.5%), amphetamine-induced psychotic disorder with hallucinations (8.5%) and attention deficit hyperactivity disorder (5.1%).

42.7% of patients consumed alcohol, 36.0% consumed nicotine and 25.3% consumed grapefruit juice in the week prior to sampling.

Regarding the positive results of the urine multidrug rapid tests, 18 (24.0%) were for marijuana, 8 (10.7%) for methamphetamines, 5 (6.7%) for amphetamines, 5 (6.7%) for benzodiazepines and 2 (2.7%) for cocaine.

The main concomitant medications were antipsychotics (20.0%), antihypertensives (17.3%), antiepileptics (14.7%), anxiolytics (13.3%), antiulcer drugs (5.3%), non-steroidal anti-inflammatory drugs (6.7%) and antidiabetics (6.7%). Whereas the main comorbidities were hypertension (18.6%), epilepsy (13.6%), type II diabetes mellitus (10.2%) and hypothyroidism (6.8%).

Genotyping

Ten polymorphisms of the main sertraline metabolizing enzymes were determined in the patients. The results of genotypic and allelic frequencies are shown in Table 2.

Additionally, from the genotypes for CYP2D6 and CYP2C19, translation of the phenotypes of these alleles was performed according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) selective serotonin reuptake inhibitors dosing guideline.[30] The frequencies of each genotypes and phenotypes determined are shown in Table 3.

Population pharmacokinetic analysis

A one-compartment model with first-order absorption and linear elimination kinetics adequately described sertraline plasma concentrations. The subroutines ADVAN2 and TRANS 2 were implemented.

Only the CL was estimated in the developed model, Ka was fixed to 0.855 1/h [28] and V was fixed to 20.2 L/kg, which was the value that provided the best prediction and the lowest OFV for this parameter.

In the base model, the typical population value of CL was estimated at 57.1 L/h and the IIV (CV%) had a value of 38.2% and was exponentially modeled. RV was best described by a proportional error model and was estimated at 0.29% with a mean sertraline concentration of 44.46 ng/mL.

On forward inclusion, the covariates that proved to have statistically significant effect ($p < 0.05$) on CL were CYP2D6*2 (Δ OFV = -4.801) and CYP2C19 phenotype (Δ OFV = -6.515). After backward elimination, both variables reached statistical significance ($p < 0.01$), therefore were included in the final model CYP2D6*2 (Δ OFV = +7.225) and CYP2C19 phenotype (Δ OFV = +6.630). The inclusion of these variables in the final model allowed explaining 11.3% of the IIV in the sertraline CL. No decrease in the RV of sertraline concentrations was observed in the final model.

Figure 1 shows the goodness-of-fit plots for the final model, where it is observed that the inclusion of the covariates in the model reduces the scatter of points around the identity line. The conditional weighted residuals (CWRES) were distributed around zero randomly within ± 3, indicating a good prediction fit of the final model.

The results of the bootstrap analysis are summarized in Table 4. These results showed that the parameters are estimated with reasonable accuracy as the parameters of the final model were found to be within the 2.5 and 97.5 percentiles of the bootstrap replicates.

The VPC showed that the variability was appropriately estimated in the final model as most of the observed values are within the confidence regions predicted by the model (90% CI) for the median and the 5th and 95th percentiles as shown in Figure 2. These results confirm that the final model is adequate to simulate concentrations over time.

Simulations of trough plasma concentrations of sertraline were performed considering the variables that influenced the model. Initially, a standard dose of 50 mg every 24 h was simulated for each of the six treatment groups that were established. Subsequently, doses of 75, 100, 150 and 200 mg every 24 h were simulated. The results of the simulations are shown in Figure 3.

Based on the results of the simulations, the most appropriate dosing regimen was determined for each of the treatment groups in which the highest number of simulated concentrations within the therapeutic range of sertraline (10 - 150 ng/mL) were achieved. The proposed dosing regimens for sertraline are summarized in Table 5.

Discussion

Psychiatric and substance use disorders account for 7.4% of the global burden of disease.[31] Treatment of these disorders represents a challenge for public health systems as adherence to antidepressant therapy is estimated at less than 30%[32] and approximately 40% of patients treated with selective serotonin reuptake inhibitors do not achieve a good treatment response.[24]

There is a high interindividual variability in the pharmacokinetics of sertraline,[33] therefore, identifying and quantifying the factors that contribute to this variability allows the determination of dosing regimens adapted to the characteristics of each individual that contribute to improved treatment response.

In this study, the pharmacokinetics of sertraline was described by a one-compartment model with first-order absorption and elimination, which is consistent with previous studies.[15,17,26,27] Due to the high variability that did not allow an adequate estimation of V, it was decided that the only pharmacokinetic parameter estimated was CL, which had a value of 66 L/h in the final model. This value obtained is in accordance with the values reported for this parameter in previous population pharmacokinetic studies (36.3 - 130 L/h).[15,17,26–28]

The covariates that were shown statistical significance and were included to describe IIV in the sertraline CL were the presence of CYP2D6*2 polymorphism (*1/*2 or *2/*2 genotype) and the CYP2C19 phenotypes.

The inclusion of these variables to the model allowed explaining 11.3% of the IIV in sertraline CL, this value is lower than other studies (19.0 - 70.1%),[15,17,26] however, this is the first time that the influence of both variables on sertraline pharmacokinetics is described. On the other hand, although there was no decrease in RV during model development, the estimated residual error (0.31%) is lower than the values reported in other pharmacokinetics population models (11.70% - 48.47%).[17,26,27]

Several studies have indicated the relevance of CYP2D6 genotype and CYP2C19 phenotype in the pharmacokinetic variability of selective serotonin reuptake inhibitors, including sertraline.[20,21,24,30,34,35]

The CYP2D6*2 polymorphism is a functional allele encoding an enzyme with slightly reduced activity (~80% of the wild-type).[36] In our study, the presence of the CYP2D6*2

polymorphism caused a 23.1% decrease in the CL of sertraline with respect to patients with wild-type genotype. The allele frequency observed was 26.3%, which is very similar to the frequencies reported for mestizo Mexican population (19.34%),[36] Mexican-Americans (18-22.8%),[37,38] Spaniards (22%)[39] and Caucasians (28.5%).[40]

Metabolism by the CYP2C19 enzyme is the main metabolic pathway for sertraline,[20,30] and it has been identified that the main alleles found in patients are CYP2C19*1, *2 and *17[30]. The CYP2C19*1 allele encodes an enzyme with normal function, the CYP2C19*2 polymorphism is a null allele encoding an inactive enzyme, while the CYP2C19*17 polymorphism results in an enzyme with increased activity.[41]

For the CYP2C19 polymorphisms we found the same frequency for both (7.6%). In the case of CYP2C19*2, the allele frequency is similar to that previously reported in mestizo Mexican population (6.9 - 8.61%),[42,43] Mexican Americans (9.7%),[44] Bolivians (7.8%)[45] and Colombians (8.7%).[46]

On the other hand, the distribution of CYP2C19*17 allele frequency shows multi-ethnic variability as low frequencies in Asian population (3%) and higher frequencies in African (16%), American (18%) and European (21%) populations.[47] Studies in Mexican mestizo and Hispanic American populations have reported frequencies of 14.29% and 12%, respectively.[43,48] These differences between the frequency found in the study (7.6%) and those previously reported in Mexican population may be due to the number of patients included in the study.

The CYP2C19 phenotypes that were identified in the patients were EM, IM, RM and PM. In order to facilitate the pharmacokinetic analysis, the RM patients were considered within the EM group, because the sertraline CL values in these groups were very similar (1%

difference) and showed no significant difference ($p > 0.05$). Therefore, the proposed dosage for EM patients can be applied to RM patients.

Several studies that have evaluated the influence of CYP2C19 phenotypes on sertraline pharmacokinetics have reported decreases in sertraline CL for IM between 14.95% - 17.84%[22,24] and decreases between 29.04% - 43.87% for patients with PM phenotype.[20,22] Our results are similar to those previously reported, since IM patients had a 19.06% decrease in sertraline CL and PM patients had a 48.26% decrease in sertraline CL, with respect to EM patients.

The simulations performed allowed to identify that with the standard sertraline dose of 50 mg/day, the probability that patients with IM, EM and EM+CYP2D6*2 phenotype do not reach concentrations within the therapeutic range is high, with percentages of patients outside the therapeutic range between 49 - 84%. Therefore, it was necessary to perform dose adjustments according to the genotype and phenotype of the treatment groups.

The recommended doses for patients with PM phenotype (75 to 100 mg/day) are in accordance with the doses proposed by the Dutch Pharmacogenetics Working Group which recommends not to exceed 75 mg/day,[41] and also agree with the observations made by Strawn et. al. who evaluated that with maximum doses of 100 mg/day, PM patients reach concentrations similar to EM.[22] In addition, because PM patients achieve higher sertraline concentrations, the CPIC recommends start treatment with a 50% reduction of the initial dose and titrate according to response.[30]

The recommended doses for the other CYP2C19 phenotypes are similar to those recommended by previous studies, for IM (150 mg/day vs 125 mg/day), EM (200 mg/day vs 150 mg/day) and RM (200 mg/day vs 200 mg/day) patients.[22]

The identification of the UM phenotype was not possible in any of our patients, however, CPIC does not recommend any dose adjustment for UM patients, however, if the patient does not respond adequately to maintenance doses of sertraline, an alternative selective serotonin reuptake inhibitors drug that is not metabolized by CYP2C19 should be considered.[30]

Our data suggest that, if CYP2D6 genotype is taken into consideration in addition to CYP2C19 phenotype, sertraline doses should be reduced for each respective group. For example, in PM patients with CYP2D6*2 polymorphism the recommended dose is 50 - 75 mg/day, for IM patients 75 - 100 mg/day and, for EM and RM patients, doses of 150 mg/day are recommended.

Some of the limitations of the study should be mentioned, we were unable to estimate pharmacokinetic parameters such as Ka and V because most of the samples obtained were in the elimination phase. In addition, it was not possible to characterize other variables that showed influence on sertraline pharmacokinetics, which may be due to the small number of patients included. Most patients did not attend the second follow-up appointment, which may not have allowed us to relate sertraline plasma concentrations to scores on the Beck's Anxiety Inventory and Beck's Depression Inventory. Adjustments to the recommended sertraline doses have not been externally validated in a similar population, therefore, should be evaluated in further studies and should be monitored in clinical practice to verify the improvement in the response to sertraline treatment.

Conclusions

A pharmacokinetic model of sertraline in dual pathology patients was developed that demonstrated adequate accuracy in estimating trough sertraline concentrations. To our

knowledge, this is the first study that characterizes both the influence of CYP2D6*2 polymorphism and CYP2C19 phenotypes on sertraline clearance. Dosing regimens were established considering these covariates with the aim of reaching therapeutic levels that contribute to improved treatment response.

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Data sharing

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Conflicting Interests

The authors declare no conflicts of interest.

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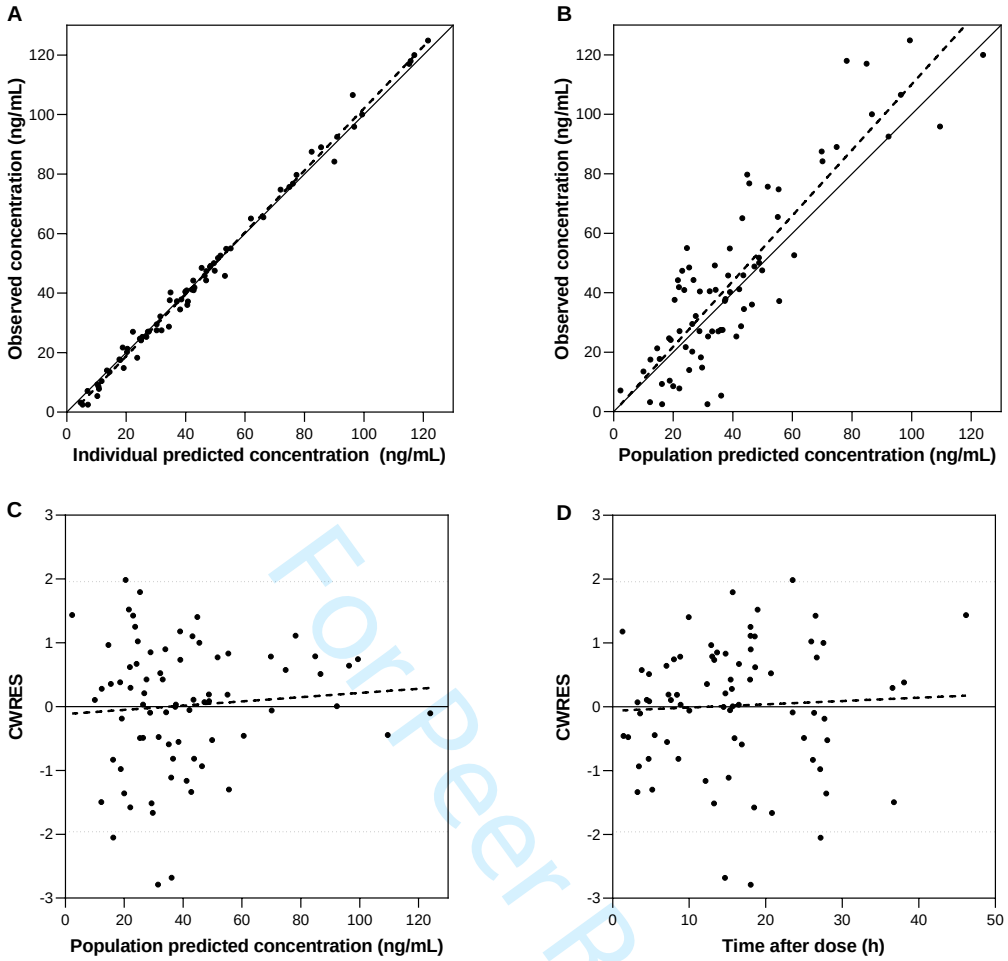
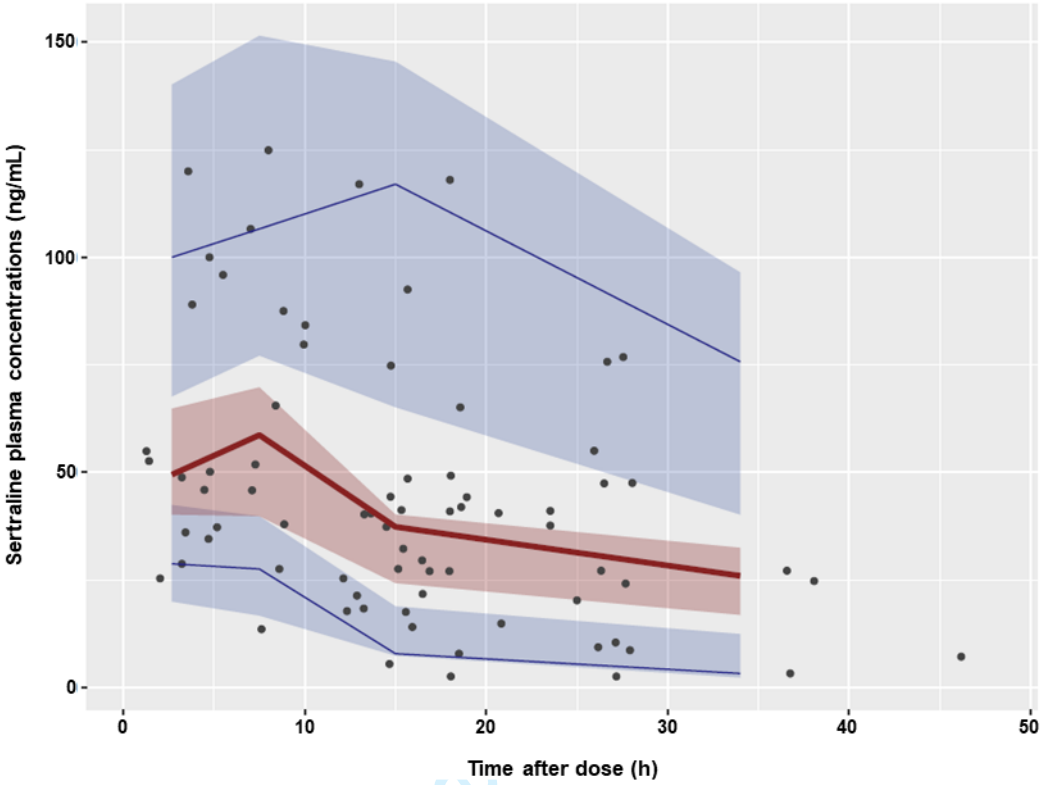
Figure legends

Figure 1. Goodness of fit plots. (A) Observed versus individual predicted concentrations. (B) Observed versus population predicted concentrations. (C) Conditional weighted residuals versus population predicted concentration. (D) Conditional weighted residuals versus time after dose.

Figure 2. Visual predictive check of the final model. Individual points represent observed concentrations. Red solid line represents 50th percentiles of observed data. Blue solid lines represent 5th and 95th percentiles of observed data. The red area represents 95% CI of the 50th percentile of predicted data. The blue areas represent 95% CI of the 5th and 95th percentiles of predicted data.

Figure 3. Simulated trough plasma concentrations of sertraline. 2D6*2: CYP2D6*2 polymorphism, PM: poor metabolizer, IM: intermediate metabolizer, EM: extensive metabolizer. (A) Standard sertraline dose of 50 mg/day. (B) Proposed dose for each group treatment: 2D6*2+PM (75 mg/day), PM and 2D6*2+IM (100 mg/day), IM and 2D6*2+EM (150 mg/day), EM (200 mg/day). Therapeutic interval is presented in dashed line.




Sertraline plasma concentrations (ng/mL)
150
100
50
0
0
10
20
30
40
50
Time after dose (h)

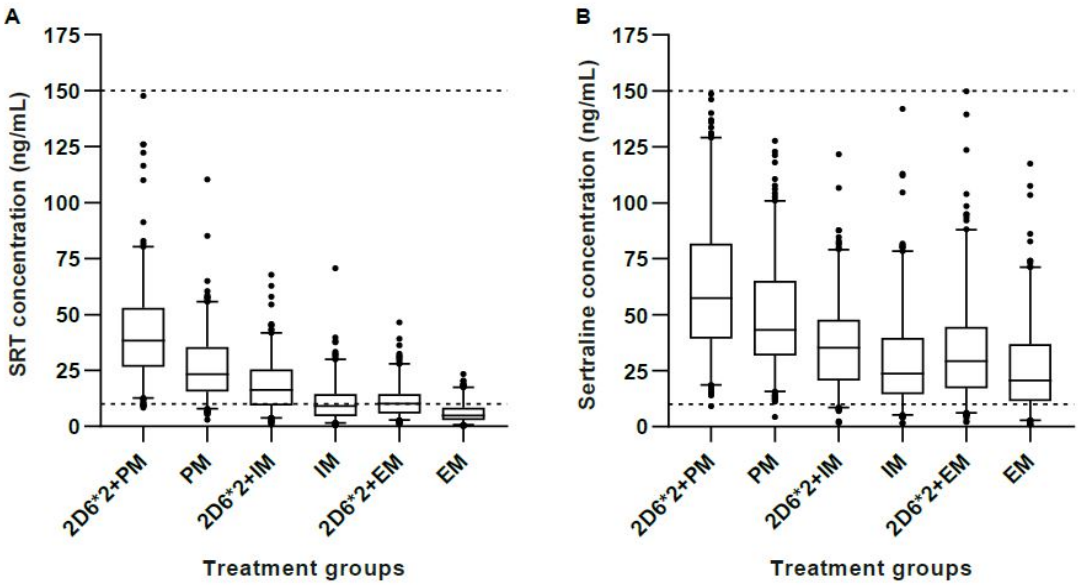


Table 1. Summary of patient characteristics (n = 59)

Patient characteristic	Value
Age (years)[a]	29 (13 - 76)
Weight (kg)[b]	71.9 ± 13.9
Height (m)[b]	1.7 ± 0.1
Body mass index (kg/m^2)[b]	26.3 ± 4.8
Glucose (mg/dL)[a]	94.2 (67 – 334)
Urea (mg/dL)[a]	24.0 (15 – 141)
Triglycerides (mg/dL)[a]	155.0 (47 – 413)
Uric acid (mg/dL)[b]	5.9 ± 1.2
Total cholesterol (mg/dL)[b]	167.2 ± 38.0
Serum creatinine (mg/dL)[a]	0.82 (0.49 – 4.18)
Creatinine clearance (mL/min)[a]	113.0 (17.6 – 150.9)
Albumin (g/dL)[b]	4.6 ± 0.3
Aspartate aminotransferase (U/L)[b]	38.8 ± 22.9
Alanine aminotransferase (U/L)[a]	59.4 ± 70.1
Hemoglobin (g/dL)[b]	15.1 ± 1.1
Hematocrit (%)[b]	45.9 ± 3.4
Platelets (10^3/μL)[b]	245.6 ± 57.0
Sertraline daily dose (mg/day)[a]	50.0 (12.5 – 100.0)
Sertraline concentration (ng/mL)[a]	40.2 (2.5 – 124.9)
Beck's Anxiety Inventory score[a]	11.0 (0 – 40)
Beck's Depression Inventory score[a]	8.0 (0 – 27)

[a]variable is presented as median (range); [b]variable is presented as mean ± SD; categorical data are presented as frequency (%)

Table 2. Genotype and allele frequency

Polymorphism	Genotype frequency (%)			Allele frequency[a]	
	Homozygous reference	Heterozygous	Homozygous variant	p	q
CYP2D6					
*2 (rs16947)	34 (57.6%)	19 (32.2%)	6 (10.2%)	0.737	0.263
*4 (rs3892097)	47 (79.7%)	8 (13.6%)	4 (6.8%)	0.864	0.136
*10 (rs1065852)	49 (83.1%)	8 (13.6%)	2 (3.4%)	0.898	0.102
*17 (rs28371706)	58 (98.3%)	1 (1.7%)	0 (0.0%)	0.992	0.008
*41 (rs28371725)	57 (96.6%)	1 (1.7%)	1 (1.7%)	0.975	0.025
CYP2C9					
*2 (rs1799853)	56 (94.9%)	3 (5.1%)	0 (0.0%)	0.975	0.025
*3 (rs1057910)	54 (91.5%)	5 (8.5%)	0 (0.0%)	0.958	0.042
CYP2C19					
*2 (rs4244285)	51 (86.4%)	7 (11.9%)	1 (1.7%)	0.924	0.076
*17 (rs12248560)	50 (84.7%)	9 (15.3%)	0 (0.0%)	0.924	0.076
CYP2B6					
*9 (rs3745274)	30 (50.8%)	29 (49.2%)	0 (0.0%)	0.754	0.246

[a]Hardy-Weinberg notation for allele frequencies: p, frequency of reference allele; q, frequency of variant allele

Table 3. Summary of CYP2D6 and CYP2C19 genotype and phenotypes

Genotype	Phenotype	Frequency (%)
CYP2D6		
*1/*1	Normal metabolizer	22 (37.3%)
*1/*2	Normal metabolizer	17 (28.8%)
*4/*10	Intermediate metabolizer	6 (10.2%)
*2/*2	Normal metabolizer	3 (5.1%)
*4/*4	Poor metabolizer	3 (5.1%)
*1/*10	Normal metabolizer	1 (1.7%)
*2/*4	Intermediate metabolizer	1 (1.7%)
*2/*41	Normal metabolizer	1 (1.7%)
*2x2/*10	Normal metabolizer	1 (1.7%)
*2x2/*17	Ultrarapid metabolizer	1 (1.7%)
*2x2/*41x2	Ultrarapid metabolizer	1 (1.7%)
*4/*10x2	Intermediate metabolizer	1 (1.7%)
*4x2/*10x2	Intermediate metabolizer	1 (1.7%)
CYP2C19		
*1/*1	Normal metabolizer	44 (74.6%)
*1/*17	Rapid metabolizer	7 (11.9%)
*1/*2	Intermediate metabolizer	5 (8.5%)
*2/*17	Intermediate metabolizer	2 (3.4%)
*2/*2	Poor metabolizer	1 (1.7%)

Table 4. Bootstrap results

			Bootstrap		
Parameter		**Mean (RSE)**	**Median**	**2.5th**	**97.5th**
Ka (h^{-1})	θ_1	0.855[a]	--	--	--
CL (L/h)	θ_2	66	66	58.3	75.6
V (L/kg)	θ_3	20.2[a]	--	--	--
$2D6*2_{(*1/*2 \text{ or } *2/*2)}$	θ_4	-0.231 (28%)	-0.227	-0.356	-0.078
PM_{2C19}	θ_5	-0.483 (7%)	-0.483	-0.543	-0.422
IM_{2C19}	θ_6	-0.191 (31%)	-0.192	-0.316	-0.066
IIV on CL (%CV)	ω^2_{CL}	33.90 (12%)	33.16	25.49	41.71
RV (%CV)	σ	0.31 (27%)	0.31	0.23	0.41

$CL (L/h) = \theta_2 * (1 + \theta_4 * CYPCD6*2) * (1 + \theta_5 * PM_{2C19}) * (1 + \theta_6 * IM_{2C19})$ $V = \theta_3 * weight$

[a]fixed value; RSE: relative standard error; Ka: Absorption rate constant; V: volume of distribution; CL: clearance; 2D6*2: CYP2D6*2 polymorphism; PM_{2C19}: poor metabolizer of CYP2C19; IM_{2C19}: intermediate metabolizer of CYP2C19; IIV: interindividual variability; RV: residual variability

Table 5. Dosage recommendations

Treatment group	CL (L/h/kg)	Sertraline dose (mg/ day)
CYP2D6*2 + $PM_{CYP2C19}$	0.365	50-75
$PM_{CYP2C19}$	0.475	75-100
CYP2D6*2 + $IM_{CYP2C19}$	0.571	75-100
$IM_{CYP2C19}$	0.743	150
CYP2D6*2 + $EM_{CYP2C19}$	0.706	150
$EM_{CYP2C19}$	0.918	200

PM: poor metabolizer; IM: intermediate metabolizer; EM: extensive metabolizer; CL: clearance.