ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 05, Issue, 08, pp. 5240-5244, August, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

UV/VIS SPECTROMETRY WITH MULTIVARIATE CALIBRATION AS A NEW APPROACH FOR THE CHIRAL ANALYSIS OF FLUOXETINE

***Roman Poláček and Pavel Májek**

Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic

ARTICLE INFO

Article History:
Received 23rd May, 2015
Received in revised form
30th June, 2015
Accepted 17th July, 2015
Published online 31th August, 2015

Key Words:
Chiral analysis,
Fluoxetine Enantiomers,
Cyclodextrins,
Multivariate Calibration.

ABSTRACT

Nowadays, a large number of chiral analyses are needed, simple and rapid methods for the determination of the enantiomeric composition in pharmaceutical products should be developed. The fluoxetine belongs to the most prescribed antidepressant chiral drugs and its enantiomers have a different duration of serotonin inhibition. This study presents cheap and fast alternative to traditional chiral techniques for the determination of the fluoxetine enantiomeric composition by the combination of UV/VIS spectrometry and multivariate calibration. The chiral recognition of the fluoxetine was based on the creating of the diastereomeric complexes with *α*- and *β*-cyclodextrin. Multivariate calibration methods, including principal component regression (PCR) and partial least square method (PLS), were used for spectral data evaluation. Small differences in results between eachcyclodextrin and both calibration models were obtained by multivariate calibration. PLS model for determination of the enantiomeric composition of fluoxetine by *β*-cyclodextrin as a chiral selector had slightly better prediction than others models. Predicted values of the enantiomeric composition in the synthetic samples are similar to their nominal values.

INTRODUCTION

Each enantiomer of the chiral drug has different pharmacokinetic, pharmacological and psychological properties. One enantiomer of a chiral molecule may be therapeutically active the other one may be inactive or may have toxic effects (Aboul-Eneinand Wainer, 1997). Therefore, it is the great interest on the determination of the enantiomeric composition in pharmaceutical industry. Chiral analysis is traditionally performed by chiroptical methods, namely polarimetry, optical rotatory dispersion, Raman optical activity and circular dichroism (Horvát *et al*., 1997; Gergely, 2000; Konno *et al*., 2013; Shen *et al*., 2014). These methods use interactions between the stereogenic center of the chiral molecules and polarized light. Non-chiroptical methods include separation technique such as chromatography (gas or liquid) or capillary electrophoresis (Mohr *et al*., 2012; Suliman and Elbashir, 2012; Douša *et al*., 2013). Chiral analysis performed by non-chiroptical methods requires the diastereomeric complex to be formed between enantiomer and chiral selector (Sullivan, 1978; Finn, 2012).

****Corresponding author: Roman Poláček***
Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovak Republic

For effective enantiomeric differentiation, the interaction occurs between the stereogenic center of the optically active molecules and the chiral center of selector. Cyclodextrins (CDs) are macrocyclic sugar molecules that have been widely used as chiral selectors due to their capability to form inclusion complex by non-covalent interaction between the molecule and the CD cavity. The evidence of creating diastereomeric inclusion complexes between CDs and a various chiral molecules in molecular spectra with the chemometric data evaluation could find in (Busch *et al*., 2004; Fakayode *et al*., 2006; Fakayode *et al*., 2009; Valderrama and Poppi, 2009; Valderrama *et al*., 2010). Chemometric tools must be used due to small spectral differences among complexes of enantiomers with various CDs. The small spectral changes are then assigned to the known enantiomeric composition of the guest analyte using standard multivariate regression modeling technique such as principal component regression (PCR) and partial least square method (PLS) (Wise *et al*., 2006). PCR and PLS were widely used for multivariate calibration in molecular spectrometry because they allow a large number of predictor variables and tolerate correlation between predictor variables in the original measured spectral data. These multivariate methods are usually compared due to their different way in transformations of original matrix to new

matrix. PCR only considers the spectral data into the transformation process, while PLS actively involves both spectral and concentration data in performing the transformation (Wise *et al*., 2006). A detail on PCR method was referred in earlier papers (Wise *et al*., 2006; Rajalahti and Kvalheim, 2011) and more information about PLS was in (Wold*et al*., 2001; Wise *et al*., 2006; Rajalahti and Kvalheim, 2011). Fluoxetine, ((±)–*N*–methyl–*γ*–[4–(trifluoromethyl) phenoxy] benzene propanamine) (FLX), belongs to the most prescribed stereospecific antidepressant drug which has very high therapeutic and commercial potential.

It is a selective serotonin reuptake inhibitor in presynaptic neurons (Grodner and Sitkiewicz, 2013). Its enantiomers have a different duration of serotonin inhibition. (*S*)-form of FLX is approximately 1.5 times more potent than (*R*)-enantiomer and displays a threefold longer duration of action (Yee *et al*., 2000). FLX is used to treat mental depression, obsessive–compulsive disorder, nervous bulimia, and premenstrual dysphoric disorder (Tabrizi and Rezazadeh, 2012). LC or GC methods with chiral columns or chiral additives into mobile phase are typical for the determination of the FLX enantiomers. Several analytical methods with chiral HPLC and chiral GC methods were published in (Lerena *et al*, 2003; Li *et al*., 2004; Mifsud and Sqhendo, 2012; Yu*et al*., 2012; Ribeiro *et al*., 2013). Only few studies described analysis of FLX enantiomers using non-chromatography methods such as capillary electrophoresis (Asensi-Bernardi *et al*., 2013) and NMR (Ali *et al*., 2005; Shamsipur *et al*., 2007). In the above-described methods are required expensive chiral columns, large amount of solvents and much analytical time which increases the cost of the chiral analysis. The advantages of molecular spectroscopy methods are rapidity and simplicity. The methods do not have also problems with co-elution and adsorption of fairly large molecules of chiral analytes on the columns walls. The aim of the present work is to show the possibility of the determination of FLX enantiomeric composition by UV spectroscopy and multivariate calibration methods. To find optimal CD for the creation of complex with FLX enantiomers multivariate models of each complex with *α*- or *β*- CD were compared. PCR and PLS methods were applied and compared to find the best regression model for the precise recognition of FLX enantiomers in the chiral mixture.

MATERIALS AND METHODS

Reagent and sample

Analytical reagent grade chemicals and doubly distilled water were used throughout this study. (*R*)-FLX, (*S*)-FLX and native CDs, *α*- and *β*-CD, pure samples were purchased from Sigma-Aldrich (United State). The stock solutions of (*R*)-FLX and (*S*)-FLX (3 mM L^{-1}) were prepared by dissolving 1 mg of each enantiomer in water. The *α*-CD and *β*-CD solutions were prepared daily by weighing appropriate portion of individual CD to obtain 3 mM L^{-1} in 50 mL volumetric flasks.

Apparatus and software

UV measurements were performed by UV/VIS spectrometer (Thermo Scientific), repeated three times at ambient temperature. The scan speed was 200nm min^{-1}. Samples were placed in 1.0 cm long quartz cuvette. Absorption spectral data acquisition used 1 nm resolution from 200 to 300 nm. All calculations were performed by Microsoft Office Excel 2010, Statistica version 12.0 (StatSoft, USA, 2013), MATLAB 8.1 (The MathWorks Inc., USA, 2013) and PLS_Toolbox version 7.9 (Eigenvector Research Inc., USA, 2014).

Analytical figure of merit

A net analyte signal (NAS) defined as the part of measured signal unique for the consider enantiomer is used for the characterization of qualities of multivariate calibration models. NAS values are utilized to the estimation of the figure of merit in calibration models using equations described in the earlier study (Ferré and Faber, 2003),but not described in detail here. The root mean square percent relative error (RMS%RE) is a useful figure of merit for quantitatively expressing the predictive efficiency of the models (Williams *et al*., 2006).

RESULTS AND DISCUSSION

Fig. 1 shows the UV absorption spectra of both individual FLX enantiomers and their inclusion complexes with *α*-CD (A) and *β*-CD (B). FLX enantiomers have one strong absorption band at about 226 nm and small absorption peaks in the range of 250-280 nm. As seen, changes of absorbance pointed out the interactions between FLX and CDs. After interaction with the CDs, (*S*)-FLX had a higher value of absorbance at λ_{max} around 226 nm than (*R*)-FLX, while in the region from 250 to 280 nm it was conversely. Different interactions and bindings between FLX enantiomers and the type of CDs enable a creation of various strong diastereomeric inclusion complexes. The cavity size of the chiral selector plays important role in the efficiency of enantiomeric interactions with CD It can assume that FLX enantiomers have weaker interactions with *α*-CD than *β*-CD because *α*-CD have smaller cavity and one sugar unit less for providing spherical interactions. On the base of the spectral changes and the differences of absorbance values it is possible to say that using *β*-CD for the determination of the enantiomeric composition of the FLX gives better prediction than using *α*-CD as a chiral selector.

Multivariate Analysis

The Fig. 1 shows overlapped absorption spectra of the investigated enantiomers where the values of absorbance at λ_{max} are not appropriate for chiral spectral analysis. Due to complexity in spectral data, more powerful multivariate calibration methods were performed. The small spectral changes and fluctuations of the absorbance among inclusion complexes are possible to describe by multivariate calibration models. The calibration models of multivariate regressions were created from spectral data of UV/VIS spectrometry. Multivariate calibrations involve a calibration, cross-validation and prediction steps. To determine the enantiomeric composition of the FLX, 50 samples were prepared for both proposed methods. These samples contained a fixed total FLX guest concentration and a fixed CD host concentration. The range of mole fraction (*R*)- and (*S*)-FLX was from 0.0 to 100.0 % with interval of 2.0 %. All calibration models were built on the spectral data obtained from the measurement of 31 samples and next 19 samples were used for cross–validation of the

models. Autoscale pre-processing with a leave-one-out cross-validation was performed for calibration, prediction and sample datasets.

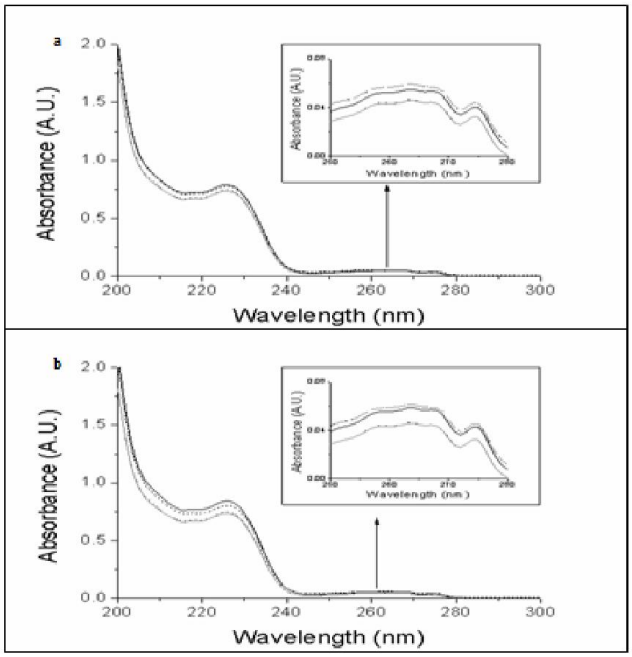

Fig.1. Measured UV/VIS absorption spectra of pure enantiomers of FLX and their complexes with *α*-CD (a) and *β*-CD (b). (*S*)-FLX (black dot line), (*R*)-FLX (gray solid line); (*S*)-FLX with CD (black solid line); (*R*)-FLX with CD (black dash line)

The selection of suitable CD and multivariate calibration method were performed by model characteristics and comparison of the figure of merit, calculated for each model. The obtained values are in Table 1. The comparison of PCR and PLS regression models was based on following characteristics calculated for each model: the root mean square error of the calibration (RMSEC), the root mean square error of cross-validation (RMSECV) and the root mean square error of prediction (RMSEP). As is shown in Table 1, the calibration models of both chiral selectors provide very similar characteristics of model and results. These calibration models consisting of three latent variables have high percent of the variance explanation of original data and relatively small values of the RMSEs. R^2 prediction values were very close to 1, what show the similarity between predicted and known values. The characteristics mentioned above denote suitability of the PCR and PLS regressions for the determination of FLX enantiomeric composition.

The results for the figure of merit for each model are also shown in Table 1. Comparison of the values of the RMS%RE for *α*-CD with those for *β*-CD indicates that inclusion complexes with *β*-CD exhibited lower values than complexes with *α*-CD. It indicates stronger interactions of FLX enantiomers with *β*-CD than *α*-CD. A little better values of the RMS%RE were obtained by PLS models than by PCR models. Calibration models provided good results for sensitivity and analytical sensitivity taking into account the analytical range

Table 1. Model characteristics and figure of merit

	α-cyclodextrin				*β*-cyclodextrin			
	PLS		PCR		PLS		PCR	
	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-
Number of latent variables	3	3	3	3	3	3	3	3
% of variancespectral block	99.85	99.85	99.85	99.85	99.91	99.91	99.91	99.91
% of variance concentration block	99.68	99.68	99.68	99.68	99.85	99.85	99.83	99.83
RMSEC[a]	0.516	0.516	0.519	0.519	0.409	0.409	0.405	0.405
RMSECV[b]	0.672	0.672	0.672	0.672	0.514	0.514	0.581	0.581
RMSEP[c]	0.669	0.669	0.673	0.673	0.581	0.579	0.575	0.575
Prediction Bias	0.313	-0.313	0.318	-0.318	0.238	-0.238	0.221	-0.245
R^2 calibration	0.997	0.997	0.997	0.997	0.998	0.998	0.998	0.998
R^2 cross-validation	0.991	0.991	0.991	0.991	0.995	0.995	0.995	0.994
R^2 prediction	0.978	0.978	0.978	0.978	0.993	0.993	0.993	0.993
RMS%RE[d]	1.995	1.957	2.506	2.661	0.798	0.797	1.582	1.549
Sensitivity	5.038	5.038	5.038	5.038	3.971	3.971	3.934	4.007
Analytical sensitivity^{-1}	6.317	6.318	3.186	3.253	1.991	2.029	1.569	1.506

[a] RMSEC - the root mean square error of calibration, [b] RMSECV - the root mean square error of cross-validation,
[c] RMSEP - the root mean square error of prediction, [d]RMS%RE - the root mean square percent relative error

Table 2. Results from the chiral analysis of the fluoxetine enantiomeric mixture

number of sample	enantiomeric composition (nominal value in %)		Determined values (%)							
			α-cyclodextrin				*β*-cyclodextrin			
			PCR		PLS		PCR		PLS	
	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-
1	50.67	49.33	48.97	51.02	49.52	50.48	49.44	50.56	50.31	49.69
2	50.00	50.00	49.06	50.83	49.17	50.88	50.42	49.58	50.41	49.59
3	49.53	50.47	48.27	51.52	48.60	51.41	49.94	50.06	49.96	50.04
			Recovery (%)							
			α-cyclodextrin				*β*-cyclodextrin			
			PCR		PLS		PCR		PLS	
			(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-	(*R*)-	(*S*)-
			96.66	103.42	97.74	102.32	97.59	102.48	99.29	100.73
			98.11	101.66	98.33	101.75	100.84	99.15	100.82	99.18
			97.45	102.09	98.10	101.86	100.82	99.19	100.86	99.16

used as chiral selector. This supports the claim that the β-CD is more appropriate to use as host molecule than α-CD although differences between interactions with analyte and chiral recognition are small. Comparison of PCR with PLS shows that PCR models had lower values of the analytical sensitivity, while the sensitivity is higher than that of PLS models. Sensitivity expresses a minimum concentration difference, which is discernible by the analytical method considering a perfect fit of the model. Analytical sensitivity is optimistic estimate which does not take into account the spectral noise. Therefore sensitivity has usually more weight in assessing the suitability of the model. The calculated PCR and PLS calibration models were used to determine the enantiomeric composition of the FLX in the racemic mixtures. Predicted enantiomeric ratios of synthetic samples were compared with known enantiomeric composition in these samples. All results are shown in Table 2. As expected from model characteristics and figure of merits, better prediction had calibration models constructed from spectral data obtained for diastereoisomeric complex with the β-CD. PCR and PLS calibration models provided comparable results in both cases. Recovery (%) is expressed as relationship between determined value from regression and a nominal value (Loco *et al*., 2002).

Conclusion

The UV/VIS spectral data obtained for the FLX enantiomers in the presence of α-CD or β-CD has been evaluated by PCR and PLS regression methods. To the comparison of multivariate calibration models, the model characteristics and figure of merit were used. The proposed method was validated by these characteristics. On the base of the results from calibration models is possible to assume that the differences between α- and β-CD, as a chiral selector in the determination of the enantiomeric composition of FLX, are small. The better results for β-CD than α-CD as a chiral selector for the enantiomers of FLX were achieved. Similar findings were obtained for regression methods, no significant differences in the residuals of the predictive values were found but the PLS models provided slightly better prediction. This proposed method is an interesting alternative to routine chiral analysis and control of the enantiomeric composition for the enantiomers of FLX in racemic mixture.

Acknowledgments

This publication was supported by the Competence Center for SMART Technologies for Electronics and Informatics Systems and Services, ITMS 26240220072, the Slovak Grant Agency VEGA (project No. 1/0573/14) and by Programme to support young researches of the SUT.

REFERENCE

Aboul-Enein, H.Y and Wainer, I.W. 1997. The Impact of Stereochemistry on Drug Development and Use, John Wiley, New York, USA.

Ali, S.M., Asmat, F., Maheshwari, A. and Koketsu, M. 2005. Complexation of Fluoxetine hydrochloride with β-cyclodextrin.A proton magnetic resonance study in aqueous solution.IlFarmaco, 60, pp. 445-449.

Asensi-Bernardi, L., Martin-Biosca, Y., Fornet-Herrero, E., Sagrado, S., Medina-Henández, M.J. 2013. Determination of Fluoxetine enantiomers in pharmaceutical formulations by electrokinetic chromatography–counter current technique.Biomed.Chromatogr., 27, pp. 377-381.

Busch, K.W., Swamidoss, I.M., Fakayode, S.O., Busch, M.A. 2004. Determination of the enantiomeric composition of some molecules of pharmaceutical interest by chemometric analysis of the UV spectra of guest–host complexes formed with modified cyclodextrins. Anal.*Chim.Acta*, 525, pp. 53-62.

Douša, M., Lehnert, P., Adamusová, H., Bosáková, Z. 2013. Fundamental study of enatioselective HPLC separation of tapentadol enantiomers using cellulose based chiral stationary phase mode. *J. Pharm. and Biomed. Anal*., 74, pp. 111-116.

Fakayode, S.O., Busch, M.A., Busch, K.W. 2006. Determination of enantiomeric composition of samples by multivariate regression modeling of spectral data obtained with cyclodextrin guest–host complexes—Effect of an achiral surfactant and use of mixed cyclodextrins. Talanta, 68, pp. 1574-1583.

Fakayode, S.O., Brady, P.M., Pollard, D.A., Mohammed, A.K., Warner, I.M. 2009. Multicomponent analyses of chiral samples by use of regression analysis of UV–VIS spectra of cyclodextrin guest–host complexes. Anal.Bioanal. *Chem*., 394, pp. 1645-1653.

Finn, M.G. 2002. Emerging methods for the rapid determination of enantiomeric excess.Chirality, 14, pp. 534-540.

Ferré, J., Faber, N. M. 2003. Net analyte signal calculation for multivariate calibration. *Chemomet. and Int. Lab. Sys.* 69, pp. 123-136.

Gergely, A. 2000. Polarimetry, ORD and CD spectroscopy.*J. Pharm. and Biomed. Anal.,* 4, pp. 553-561.

Grodner, B., Sitkiewicz, D. 2013. Enantiomers: a new issue in pharmacotherapy of depression? Psychiatr. Pol., 3: 511-516.

Horvát, P., Gergely, A., Noszál, B. 1997. Determination of enantiomeric purity by simultaneous dual circular dichroism and ultraviolet spectroscopy. Talanta, 44, pp. 1479-1485.

Konno, K., Shinna, I., Yui, H. 2013 Fine structures in vibrational circular dichroism spectra of chiral molecules with rotatable hydroxyl groups and their application in the analysis of local intermolecular interactions. *J. Mol. Struc*., 1035, pp. 260-266.

Lerena, A., Dorado, P., Berecz, R., González, A., Norberto, M., De La Rubia, A. 2003. Determination of Fluoxetine and Norfluoxetinein human plasma by high-performance liquid chromatography with ultraviolet detection in psychiatric patients. *J. Chromatogr. B Analyst Technol. Biomed.Life. Sci.,* 783, pp. 25–31.

Li, K., Thompson, M., Mcgregor, I. 2004. Rapid quantitation of Fluoxetine and Norfluoxetinein serum by microdisc solid-phase extraction with high-performance liquid chromatography-ultraviolet absorbance detection. *J. Chromatogr. B Analyst Technol. Biomed.Life. Sci.,* 804, pp. 319–26.

Loco, J.V., Elskens, M., Croux, Ch., Beernaert, H. 2002. Linearity of calibration curves: use and misuse of the

correlation coefficient. Accred. Qual. Assur., 7, pp. 281-285.

Mifsud, J., Sqhendo, L.J. 2012. A novel chiral GC/MS method for the analysis of fluoxetine and norfluoxetine enantiomers in biological fluids. *J. Pharm. Bioallied. Sci.,* 4, pp. 236–245.

Mohr, S., Weiss, J. A., Spreitz, J., Schmid, M. G. 2012. Chiral separation of new cathinone and amphetamine related designer drugs by gas chromatography – mass spectrometry using trifluoroacetyl–L–prolyl chloride as chiral derivatization reagent. *J. Chromatogr. A*, 1269, pp. 352-359.

Rajalahti, T., Kvalheim, O.M. 2011. Multivariate data analysis in pharmaceutics: A tutorial review. *Int. J. Pharm.*, 417, pp. 280– 290.

Ribeiro, A.R., Afonso, C.M., Castro, P.M.L., Tiritan, M.E. 2013 Enantioselective HPLC analysis and biodegradation of atenolol, metoprolol and fluoxetine, Environ. Chem. Lett. 11, pp. 83-90.

Shamsipur, M., Dastjerdi, L.S., Haghgoo, S., Armspach, D., Matt, D., Aboul-Enein, H.Y. 2007. Chiral selectors for enantioresolution and quantitation of the antidepressant drug Fluoxetine in pharmaceutical formulations by ^{19}F NMR spectroscopic method. *Anal.Chim.Acta*, 601, pp. 130-138.

Shen, J., Yang, J., Heyse, W., Schweitzer, H., Nagel, N., Andert, D., Zhu, Ch., Morrison, V., Nemeth, G.A.,Chen, T.M., Zhao, Z., Ayers, T., Choi, Y.M. 2014 Enantiomeric characterization and structure elucidation of Otamixaban. *J. Pharm. Anal.,* 4 (3), pp.197–204.

Sullivan, G.R. 1978. Chiral Lanthanide Shift reagents.Top. Stereochem., 10, pp. 2874-2879.

Suliman, F.O., Elbashir, A.A. 2012. Enantiodifferentation of chiral baclofen by b–cyclodextrin using capillary electrophoresis: A molecular modeling approach. J. Mol. Struc., 1019, pp. 43-49.

Tabrizi, A.B., Rezazadeh, A. 2012. Development of a dispersive liquid–liquid microextraction technique for the extraction and spectrofluorimetric determination of Fluoxetine in pharmaceutical formulations and human urine. *Adv. Pharm. Bull*., 2, pp. 157-164.

Valderrama, P., Poppi, R.J. 2009. Determination of propranolol enantiomers in plasma and urine by spectrofluorimetry and second–order standard addition method. Anal.*Chim.Acta*, 651, pp.31-35.

Valderrama, P., Romero, A.L., Imamura, P.M., Magalhães, I.R.S., Bonato, P.S., Poppi, R.J. 2010. Quantification of chlorpheniramine maleate enantiomers by ultraviolet spectroscopy and chemometric methods.Anal.Bioanal. Chem., 379, pp. 181-188.

Williams, A.A., Fakayode, S.O., Huang, X., Warner, I.M. 2006. Use of multivariate analysis for optimization of separation parameters and prediction of migration time, resolution, and resolution per unit time in micellar electrokinetic chromatography. Electrophoresis, 27, pp. 4127–4140.

Wise, B.M., Gallagher, N.B., Bro, R., Shaver, J.M., Windig, W., Koch, R.S. 2006. PLS Toolboox 4. 0 for use with Matlab^{TM}. Eigenvector Research Inc., Wenatchee.

Wold, S., Sjostrom, M., Eriksson, L. 2001. PLS-regression: a basic tool of chemometrics.Chemomet.and Int. Lab. Sys., 58, pp. 109-130.

Yee, L., Wong, S.H.Y., Skrinska, V.A. 2000. Chiral high-performance liquid chromatographic analysis of Fluoxetine and Norfluoxetine in rabbit plasma, urine, and vitreous humor using an Acetylated β –Cyclodextrin. *J. Anal. Toxicol.,* 24, pp. 651-655.

Yu, L., Wang, S., Jiang, H., Zhou, H., Zeng, S. 2012. Simultaneous determination of fluoxetine and norfluoxetine enantiomers using isotope discrimination mass spectroscopy solution method and its application in the CYP2C9-mediated stereoselective interactions. *J. Chromatogr.* A, 1326, pp. 97-104.

****