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New Method Development for Pregabalin Detection in Human Plasma by HPLC-DAD

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Abstract: Pregabalin, (S)-3-(amino ethyl)-5-methylhexanoic acid, is one of prescribed drugs with increasing abuse. Pregsal, ((S,E)-3-(((2-hydroxybenzylidene)amino)methyl)-5- methylhexanoic acid) is a novel pregabalin functionalized salicylaldehyde derivative. Present study aimed to development of new method for accurate detection of pregabalin in human plasma by HPLC-DAD through increasing its chromatographic sensitivity by derivatization and application of this method on human volunteers.

Separation of pregabalin derivative was done using HPLC-DAD, Agilent 1200 series, on reversed phase columns by phosphate buffer and acetonitrile. Pregabalin in the sample was drevatized using Salicylaldehyde solution with formation of pregsal. Pregsal was extracted using liquid liquid extraction, Pregsal was best extracted using dichloromethane in slightly acidic PH. The best chromatographic separation of pregsal was on Zorbax - C8 (250 mm ×4.6 mm, 5 µm) column, gradient mobile phase (phosphate buffer with triethylamine 4.5 and acetonitrile) at 0 minute 65:35and 6.1 minute 50:50 and with flow rate 1.5. Retention time for pregsal 14.1±0.3 minute and for ibuprofen 17.8± 0.3 minute. LOD of pregabalin in extracted samples was 1000 ng/ml and LOQ was 2500 ng/ml. Linearity range was from 2500 to 40000 ng/ml and r^2 was 0.988. The mean recovery of pregabalin from plasma was 73.8%.
further method validation is needed for the present method.

Keywords: HPLC-DAD, pregabalin, pregabalin derivative, pregsal.

1 Introduction

Pregabalin **Fig. (1)**, (S)-3-(amino ethyl)-5-methylhexanoic acid, an analogue of gamma amino butyric acid (GABA) with lipophilic properties, is a potent agonist of $\alpha 2\delta$ subunit of voltage dependent calcium channels**[**1].
Pregsal ((S,E)-3-(((2-hydroxybenzylidene)amino)methyl)-5- methylhexanoic acid) is a novel pregabalin functionalized salicylaldehyde derivative afforded prospective pain, inflammation, and pyrexia alleviating propensities**[**2**]**, see **Fig. (2)**.

2 Material and Methods

A-Chemicals

Pregabalin (99.6%) was supplied by Mash premiere for pharmaceutical industry, Egypt. Ibuprofen (98% (HPLC) salicylaldehyde. (98%), HPLC grade solvents (Methanol, acetonitrile and dichloromethane), Tert-butyl Methyl ether (TBME) (98%), Potassium dihydrogen phosphate, o-phosphoric acid were purchased from Sigma- Aldrich, Germany. Sodium carbonate, sodium hydroxide, sodium acetate, acetic acid and ammonia (33%) were purchased from El-Nasr pharmaceutical chemicals company, Egypt. Blank human plasma was obtained from Blood Transfusion Service, Sohag University Hospitals.

B-Instrumentation:

High Performance Liquid Chromatography (An Agilent technologies 1200 series, USA) consisting of quaternary pump, vacuum degasser, autosampler injector and photodiode array detector. Agilent ChemStation Software was used for the results. A Zorbax eclips- C18 (150 mm ×4.6 mm, 5 µm), a Zorbax - C18 (250 mm ×4.6 mm, 5 µm) (USA) and a Zorbax - C8 (250 mm ×4.6 mm, 5 µm) columns were purchased from agilent, USA.

C-Chromatograpic conditions:

Ibuprofen was found to be the chemical of choice as IS for pregabalin derivative. See **Fig. (3)**. Mobile phase for pregabalin and pregabalin derivative (pregsal) detection was optimized to determine the suitable condition that gives the best separation and peak shape. Different isocratic and gradient conditions by using acetonitrile and different PH phosphate buffer (2-7) at different ratios (30:70, 35:65, 40:60, 45:55, 50:50 and 60:40 v/v). A mobile phase pumped at different flow rate was used to elute the analytes from the column. The column was equilibrated for two

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minutes. Total run time was 20 min. Injection volume was 100 µl. The detector was set to scan from 200 to 800 nm for detection of maximum absorbance for pregabalin, pregsal, ibubrofen) and had a discrete channel set at 218 nm and 400nm. The column was equilibrated for two minutes. Total run time was 20 min. Injection volume was 100 µl.

Pregabalin concentrations of the quality controls were determined from three standard calibration curves, using peak area ratio for quantification. Sensitivity [limits of detection (LOD) and quantification (LOQ)], linearity [correlation coefficient (r^2)], recovery of pregabalin from plasma, accuracy and precision were calculated[3].

D-Chemicals preparation:

Phosphate buffer with and without triethylamine different PH (PH 3.5, PH 4.5, PH 5.5, PH 6, PH6.5 and PH 7.0) and according to[4], Acetate buffer (PH 7.0) and salicylaldehyde solution (0.3% v/v) according to[5].

E-Sample drevatization:

Drevatization reaction of pregabalin (pregsal) was modified from *Sherin and Ola 2012*[5]. One ml plasma added to one ml cold mix of acetonitrile and methanol (85:15) then cooling by freezing at -20°C for 10 min then centrifugation for five minutes at 3500 rpm. Supernatant was transferred into screw capped glass tubes then 100µl of Salicylaldehyde was added followed by 400 µl of acetate buffer (pH 7). Tubes were mixed well using a vortex mixer then heated on water bath with a temperature maintained at 70°C for 20 minutes then were cooled to room temperature.

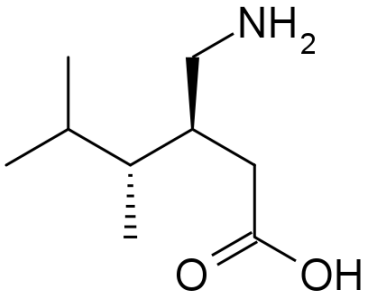

Fig (1): pregabalin.

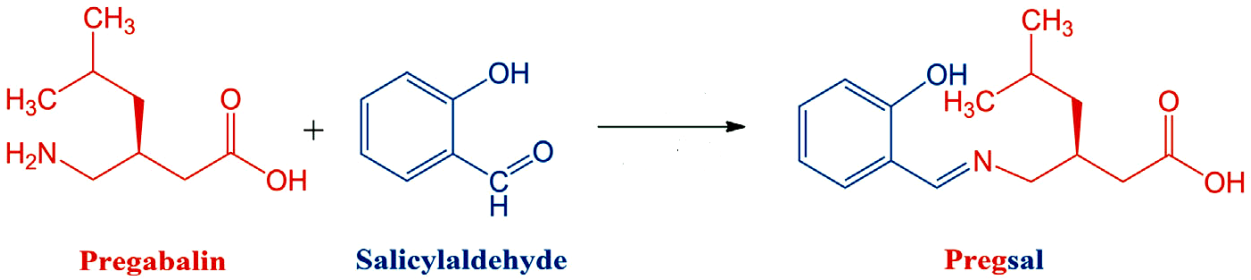

Fig (2): Synthesis of pregsal.

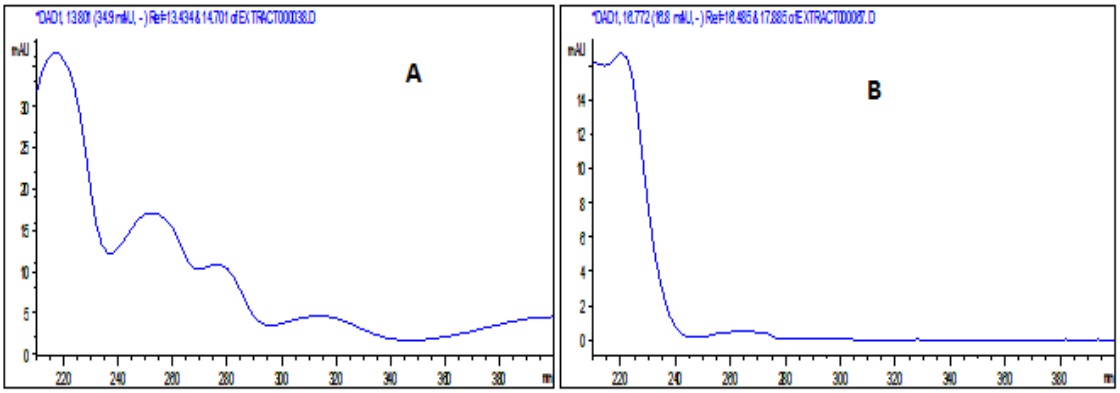

Fig (3): UV spectrum for pregsal (A), uv spectra for ibuprofen (B).

F-Extraction procedure:

Liquid/liquid extraction was performed on spiked plasma after drevatization by different solvent such as Methyl-tert-butyl ether, dichloromethane, diethyl ether and chloroform at a different PH for pregsal (ranged from PH 5 to 6.8).

G-Subjects:

Oral 150 mg tablet lyrica was administrated by five healthy volunteers (25-50 years old) from Sohag Clinical Toxicology Laboratory after approval from the ethical committee of Sohag University and informed written consent. Blood samples were withdrawn in heparinized tubes after1 hour from administration of the drug. prgabalin plasma level was tested 5 times for each sample by the present method.

3 Results

a-Chromatographic condition:

Detection was done at 218 nm. The best separation for pregsal was on Zorbax - C8 (250 mm ×4.6 mm, 5 µm) column, gradient mobile phase (phosphate buffer with triethylamine 4.5 and acetonitrile) at 0 minute 65:35and 6.1 minute 50:50 and with flow rate 1.5. LOD for extracted samples was 1000 ng/ml and LOQ was 2500 ng/ml. Retention time for pregsal 14.1±0.3 minute and for ibuprofen 17.8± 0.3minute see **Fig. (4)**.

b-Calibration:

Standard calibrators (2500, 5000, 10000, 20000, 30000, and 40000 ng/ml) and quality control (15000 and 35000 ng/ml) for pregabalin were prepared by spiking blank plasma by appropriate amount from stock standard (100 μg/ml). Working internal standard solution was containing 500 µg/ml of ibuprofen see **Fig. (5)**. Quality control and volunteers' samples measurements are shown in table (1 and 2 respectively). The mean recovery of pregabalin from plasma was 73.8%. Linearity range was from 2500 to 40000 ng/ml. Regression (r^2), mean of intercept and mean of slope for the calibration curves were 0.988,0.0015and 0.0001recepectively. . Accuracy and precision for calibrators and quality control were calculated from three calibration curves as shown in table (1,2 respectively).

c-Sample preparation:

50 µL of ibuprofen was added (500 µg/ml), followed by 100 µL (HCl 4N) then two ml of dichloromethane was added. The tubes were gently mixed for three minutes followed by centrifugation at 3500 rpm for five minutes. Lower Organic layer was transferred into an empty tube. The dried extracts were reconstituted in 150 µl of mobile phase 100 µl of the sample was injected into the HPLC – DAD system. Pregabalin plasma levels after 1 hour from administration of 150mg tablet of lyrica are shown in table (3).

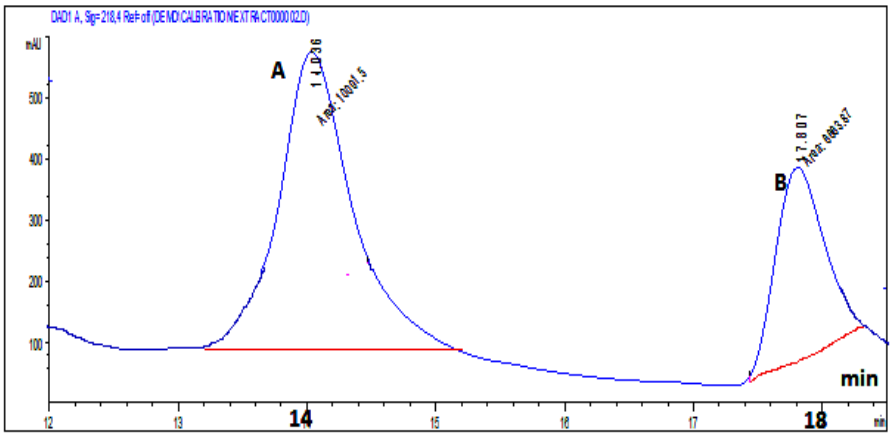

Fig (4): Retention time pregsal(A)14.1, ibuprofen(B)17.8

Table 1: accuracy and precision for calibrators calculated from three calibration curves.

Actual spiked concentration	Measured concentration		Accuracy (Bias%)	Precision (RSD%)
	Mean	SD		
2500	2682.15	50.34	7.29%	2.01%
5000	4769.39	50.32	-4.61%	1.01%
10000	11421.64	121.61	14.22%	1.22%
20000	19233.83	80.83	-3.83%	0.40%
30000	28476.09	50.32	-5.08%	0.17%
40000	43480.14	50.35	8.70%	0.13%

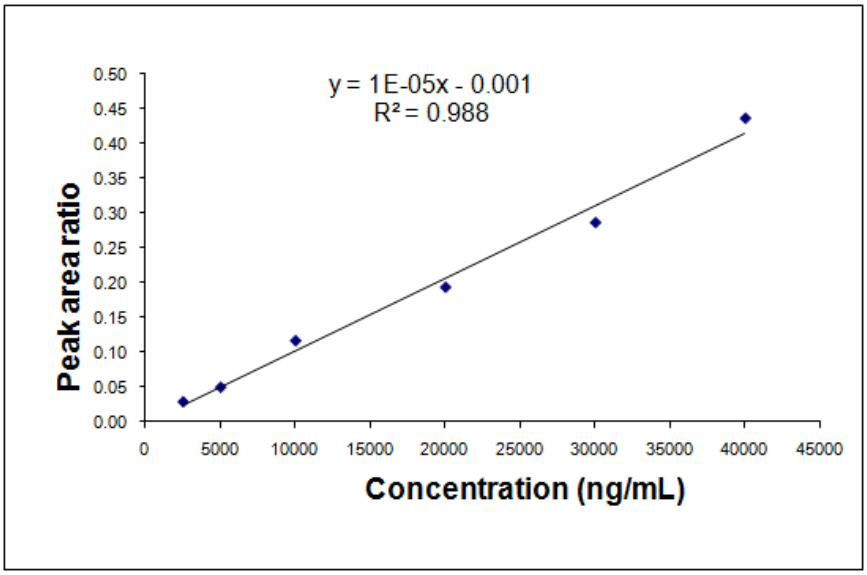

Fig (5): Calibration curve of plasma pregabalin level.

Table 2: Accuracy and precision for quality control samples calculated from nine measurements of the three calibration curves.

Actual spiked concentration	Measured concentration		Accuracy (Bias%)	Precision (RSD%)
	Mean	SD		
15000	15439.07	563.9	2.9%	3.76%
35000	35439.23	590.65	1.25%	1.69%

Table 3: Pregabalin plasma level after 1 hour from administration of 150mg tablet of lyrica

Patient		1	2	3	4	5
concentration ng/ml	Mean ± SD	3394.88 ±93.7	3550.7 ±51.3	3272.5 ±70.2	3874.5 ±83.8	3794.86 ±81.3
	Median (range)	3542.3(3199.5-4001.1)				

4 Discussions

Salicylaldehyde was used in this study to react with the primary aliphatic amine in pregabalin to form a schiff's base. Derivatization with salicylaldehyde was carried out to increase the chromatographic sensitivity and increase retention time of pregabalin[5], See **Fig. (4)**. Pregabalin is an aliphatic agent without any significant chromophore group, which makes difficulty in its quantification by general HPLC-UV methods, see **Fig. (6)**. Therefore, different studies were used derivatizing reagents for determination of Pregabalin such as Na-5-Fluoro-2,4-dinitrophenyl-5-L-alanine amide[6,7], 9-fluorenylmethyl chloroformate (FOMC-Cl) [8], 1- Fluoro- 2,4 dinitrobenzene, o-phthaldialdehyde (OPA), 3-mercaptopropionic acid, and picrylsulfonic acid[9,10,11], and fluorescamine[12,13].

liquid extraction of the derivative was in slightly acidic PH helped in considerable recovery of pregsal in organic layer. In this study, measurements for quality control and volunteers' samples as an application for the method were in agreement with previous studies.

After oral 150 mg pregabalin, Bockbrader et al.[11] found that maximum plasma concentration was at about 1.3 h, Vaidya et al.[14] reported that range of (Cmax) from was 3.5 to 4.5 µg/mL and Tafesse et al.[15] who collected Plasma samples after 1 h. Other studies for higher oral and toxic doses reported higher plasma levels such as Ahmadkhaniha et al.[16] who gave single oral dose of pregabalin tablet (400mg) (Cmax = 8.2, Tmax= 2–3 h). Kriikku et al.[17] conducted study on Individuals who had been apprehended for driving under the influence of drugs showed blood levels of pregabalin up to 111.6 mg/L.

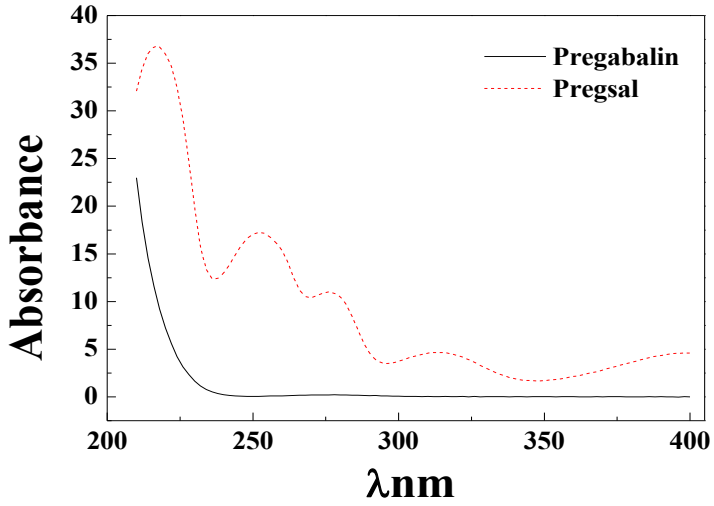

Fig (6): Uv spectrum for prgabalin and pregabalin derivative (pregsal).

5 Conclusions and Recommendation

The present method can accurately detect different plasma levels with limit of detection that can cover both therapeutic and toxic levels. Further method validation is needed for the present method.

References

[1] Rogawski M. A. and Taylor C. P. (2006): Calcium channel $\alpha 2 - \delta$ subunit, a new antiepileptic drug target. Epilepsy Research., **69(3)**, 183–272(2006).

[2] Ahmad N., Subhan F., Islam N. U., Shahid M., Rahman F. U.,and Fawad K.(2017): A Novel Pregabalin Functionalized Salicylaldehyde Derivative Afforded Prospective Pain, Inflammation, and Pyrexia Alleviating Propensities. Arch. Pharm. Chem. Life Sci., **350**, e1600365(2017).

[3] Food and drug administration (2018): Bioanalytical Method Validation Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM), Biopharmaceutics., 1-41(2018).

Abdel-Aziz Y. E , Khaled M. M. , Maha A.Hilal, Soheir A.M. , Khaled E. A. and Ahmed Y. N.(2011): Development and Validation of High-Performance Liquid Chromatography–Diode Array Detector Method for the Determination of Tramadol in Human Saliva. J Chromatograph Separat Techniq, **2(4)**: 1000114(2011).

Sherin F. H. and Ola M. A.(2012): Optimized and validated spectrophotometric methods for the determination of pregabalin in pharmaceutical formulation using ascorbic acid and salicylaldehyde. Journal of American Science, **8(12)**, 118-124(2012).

[4] Jadhav A. S., Pathare D. B. and Shingare M. S.(2007): Validated enantioselective LC method, with precolumn derivatization with Marfey's reagent, for analysis of the antiepileptic drug pregabalin in bulk drug samples.

[5] Chen X . , Zhang D . , Deng J . , and Fu X(2008).: Determination of Optical Impurity of Pregabalin by HPLC with Pre-Column Chiral Derivatization . Journal of Chromatographic Science., **46**, 42-44(2008).

[6] Bank R.A., Jansen E.J., Beekman B., and Koppele J.M.. (1996): Amino acid analysis by reverse-phase high-performance liquid chromatography: improved derivatization and detection conditions with 9-fluorenylmethyl chloroformate. Anal Biochem., **240(1)**, 167–176(1996).

[7] Vermeij T.A.C., Edelbroek P.M. (2004;): Simultaneous high-performance liquid chromatographic analysis of pregabalin, gabapentin and vigabatrin in human serum by precolumn derivatization with o-phtaldialdehyde and fluorescence detection. Journal of Chromatography B., **810**:297–303(2004).

[8] Berry D., and Millington C.(2005): Analysis of Pregabalin at Therapeutic Concentrations in Human Plasma/Serum by Reversed-Phase HPLC. Ther. Drug Monit., **27(4),** 451-456(2005).

[9] Bockbrader H. N., Radulovic L. L., Posvar E. L. (2010.), ., "Clinical pharmacokinetics of pregabalin in healthy volunteers," Th Journal of Clinical Pharmacology., **50**(8), 941–950(2010).

[10] Martinc B., Grabnar I., Mrhar A. and Vovk, T. (2010): Rapid high-performance liquid chromatography method for determination of pregabalin in a pharmaceutical dosage form following derivatization with fluorescamine. AOAC Int., **93**, 1069.

[11] Walash M. I., Belal F., El-Enany N. and El-Maghrabey M. H. (2011): Simple and sensitive spectrofluorimetric method for the determination of pregabalin in capsules through derivatization with fluorescamine. Luminescence., **26(5)**, 342-348(2011).

[12] Vaidya V. V., Yetal S. M., Roy S. M. N., Gomes N. A., and Joshi S.S.(2007): LC-MS-MS determination of pregabalin in

human plasma. Chromatographia., **66(11-12)**, 925–928(2007).

[13] Tafesse T. B., Mazdeh F. Z., Chalipour A., Tavakoli M., Hajimahmoodi M., Amini M. (2018): Gas Chromatography–Mass pectrometry Determination of Pregabalin in Human Plasma Using Derivatization Method. Chromatographia., **81**, 501–508(2018).

[14] Ahmadkhaniha R., Mottaghi S., Zargarpoor M., and Souri E. (2014): Validated HPLC Method for Quantification of Pregabalin in Human Plasma Using 1-Fluoro-2,4-dinitrobenzene as Derivatization Agent Chromatography Research International Volume, Article ID 450461, 6 pages.

[15] Kriikku P., Wilhelm L., Rintatalo J., Hurme J., Kramer J., Ojanperä I. (2014): Pregabalin serum levels in apprehended drivers. Forensic Sci. Int., **243**, 112–116(2014).