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Validated RP HPLC Method for the Determination of Related Substance of Oxcarbazepine an Antiepileptic DRUG

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Abstract: A reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the quantitative estimation of Oxcarbazepine and its impurities (related substances). The chromatographic separation was performed on a Inertsil ODS3V column, with a particle size of 5µ (250 mmX4.6 mm id.) and a mixture of 0.1% NH_4OH in water adjusted to pH 5.0with glacial acetic acid, acetonitrile as mobile phase at flow rate of 1.0 mL/min. Calibration showed that the response of impurity and Oxcarbazepine was a linear function of concentration over the range 0.25–0.75 µg/mL and 2.5-7.5 µg/mL respectively ($r^2 \geq 0.999$) and the method was validated over this range for precision, accuracy, linearity and specificity. For precision study, RSD of each impurity and drug substance was found to be in prescribed limit. The method was found to be precise, accurate, linear and specific. The proposed method was successfully employed for related substances analysis of Oxcarbazepine. A simple gradient method with 15 minutes run time for determination of all three critical parameters is an added advantage of getting quality product.
Keywords: Antiepileptic agent, Oxcarbazepine, RP-HPLC, Validation.

Introduction

Antiepileptic drug used to treat or prevent convulsions (as in epilepsy). Oxcarbazepine is an anti-epilepticprodrug with 10-monohydroxy (MHD) derivative of oxcarbazepine as an active metabolite mainly responsible for antiepileptic activity by blocking of voltage dependent sodium channels present in brain. Chemically, Oxcarbazepine is 10, 11-Dihydro-10-oxo-5*H*-dibenz [*b*,*f*] azepine-5-carboxamide[1]. Oxcarbazepine is a structural derivative of carbamazepine, with a ketone in place of carbon-carbon double bond on the dibenzazepine ring [2]. This structural modification helps to protect the liver in metabolizing the drug and prevents the serious consequences of anemia which is occasionally found with carbamazepine treatment. Hence, treatment with Oxcarbazepine helps in reduction of side effects associated with carbamazepine treatment.Literature survey revealed that spectrophotometric[3-7],chromatographic[8-21] and Voltametric[22-23] methods are available for estimation of Oxcarbazepine individually and in combination with other drugs in different formulation. Hence we attempted to develop a simple, rapid and accurate RP-HPLC method for estimation of Oxcarbazepine and its related substances.

Materials and Methods

Oxcarbazepine and all impurities were obtained from Jubilant LifeSciences Ltd. Noida, India. Acetonitrile (HPLC grade) and ammonium hydroxide (AR grade) were purchased from SD fine chemicals, Noida, Indiaanddistilled water used was prepared in laboratory.

Instrumentation

HPLC analysis was performed with Waters 2695 system equipped with a quaternary solvent manager, sample manager, column-heating compartment, UV2487 and photodiode array detector 2996. This system was controlled by empower software. The column used was Inertsil ODS3V, (250 mm, 4.6mm id.), 5 µm particle sizeemployed for chromatographic separation at column oven temperature of 35^0C with an gradient run program at a flow-rate of 1.0 mL/min. The mobile phase consists of solvent A: 0.1% NH_4OH (1 mL of NH_4OH in one liter of distilled water) the pH of this solution was adjusted to 5 with glacial acetic acid and solvent B: acetonitrile. The mobile phase was filtered through a 0.45 µm Millipore filter individually, followed by sonication and degassing for 10 minutes before use. The detection was performed at 256 nm wavelength. The sample injection volume was 20 µL and run time was 15 minutes.

Preparation of standard solutions

Stock standard solution was prepared by dissolving 25mg of Oxcarbazepine in 50mLvolumetric flask and dissolved in acetonitrile volume of made up to the mark with acetonitrile to get a solution containing oxcarbazepine500µg/mL.The working standard was prepared by diluting the above stock solution in mobile phase to reach a concentration range of 2.5-7.5 µg/mL.

Preparation of individual standard solution of impurities (Solution II)

5mg of each impurity A, impurity B, impurity C and impurity D into four separate 100mLvolumetric flask, dissolved and made volume up to mark with acetonitrile.

Preparation of impurities stock solution (Solution III)

Pipette out 5mL of each solution of Solution II in 100mL volumetric flask and dissolved and made up to mark with acetonitrile.

Preparation of test solution

25mg of Oxcarbazepine was accurately weighted and transferred into 50mL volumetric flask and dissolved with 5mL of acetonitrile. To this 10mL of mixed solution III was added and mixed and diluted to volume with acetonitrile (500µg/mL Oxcarbazepine and 0.5 µg/mL of impurity). The working test solutions were prepared by diluting the above test solution in mobile phase to reach a concentration range of 2.5-7.5 µg/mL for Oxcarbazepine and 0.25-0.75 µg/mL for impurities.

Method Validation

The method was validated for specificity, precision, accuracy, LOD, LOQ, linearity and robustness as per the International Conference on Harmonization (ICH) guidelines[24].

Specificity

A study was performed to demonstrate the interference from placebo. Sample solutions were prepared by taking the placebo equivalent to the amount present in the sample solution and analyzed as per test method. A study was conducted to demonstrate the known impurities interference by spiking the sample solution with all the known impurities at 0.1% spike level of test concentration and analyzed as per test method.The known impurities of Oxcarbazepine were injected individually to confirm the retention time.

Linearity

The linearity of the method was determined at five concentration levels ranging from 0.25-0.75 µg/mL for impurities and 2.5-7.5 µg/mL for Oxcarbazepine. The calibration curves were constructed by plotting peak areas versus concentration of impurities and Oxcarbazepine. The slope, Y-intercept and correlation coefficient were calculated.

LOD and LOQ

Sensitivity of the method was determined with respect to limit of detection and limit of quantification for Oxcarbazepine impurities. Series of lower concentration of drug solution and its impurities were injected. LOD and LOQ calculated by signal to noise ratio method. LOD => S/N =3 & LOQ => S/N =10.

Precision

The precision of Oxcarbazepine and related substances (RS) test method was evaluated by intraday and interday precision using six samples spiked with known impurities at 0.1% level.

Accuracy

To confirm the accuracy of the method, studies were carried out by standard addition technique. Related substances (RS) samples were prepared in triplicate by spiking all known impurities in test preparation at the level of 80%, 100% and 120% of the limit concentration (0.1%) and analyzed as per the chromatographic method mentioned above.

Robustness

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions such as flow rate, column temperature and wavelength of detection.

Results and Discussion

The development of an analytical method for the determination of drugs and related substances by HPLC has gain popularity in recent years because of their importance in quality control of drug products and its related substances. The objective of this study was to develop a rapid and sensitive HPLC method for estimation of Oxcarbazepine and its related substances using Inertsil ODS3V, (250 mm, 4.6mm id.), 5 µm particle sizecolumn with gradient system. The mobile phase was optimized using solvent A- 0.1% NH_4OH and solvent B-acetonitrile. From the UV spectrum of Oxcarbazepine and its related substances, wavelength was selected, at 256nm, is absorptive point for the Oxcarbazepine and its related substances. Good resolution was carried out at 256nm and Oxcarbazepine and its related substancesshowed good absorbance at this wavelength. Optimized chromatographic conditions were shown in Table 1.

Table 1: Chromatographic conditions

Mobile Phase Used	Solvent A-0.1% NH_4OH in water (pH-5.0 with GAA) : Solvent B- Acetonitrile, Gradient method
Column	Inertsil C-18 ODS-3V, (25cm×4.6 mm, 5µm)
Diluent	Acetonitrile
Temperature	35°C
Wavelength	256 nm
Injection Volume	20µL
Flow	1.0 mL/min
Run Time	15 mins

Validation of method

All parameters of proposed method were validated as per the ICH guidelines.

Specificity

The Specificity of the HPLC method was carried out. The complete separation of Oxcarbazepine in the presence of its impurities was observed. The average retention times were found to be 4.353 min. forOxcarbazepine, 5.217 min. for Impurity A, 5.898 min.for Impurity B, 8.354 min. for Impurity C, and 12.685 min forImpurity D. The peaks obtained were sharp and have clear baseline separation were shown in figure 1.

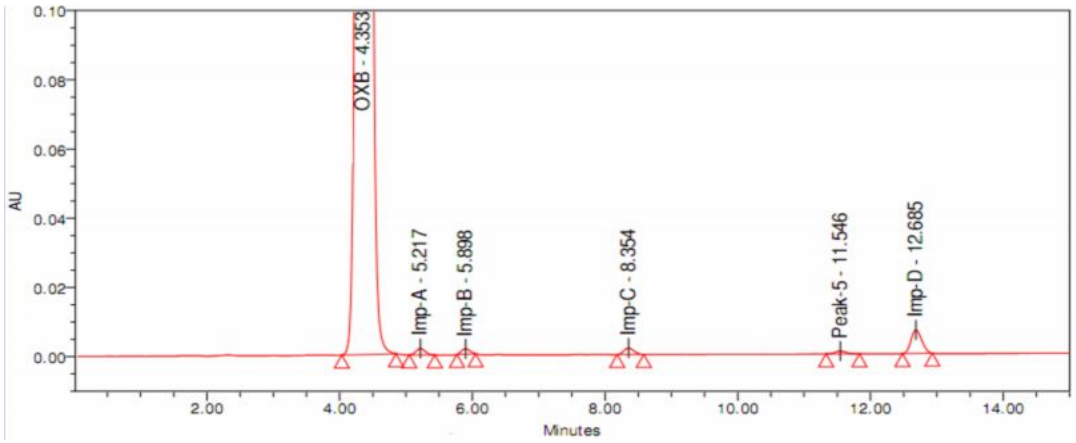
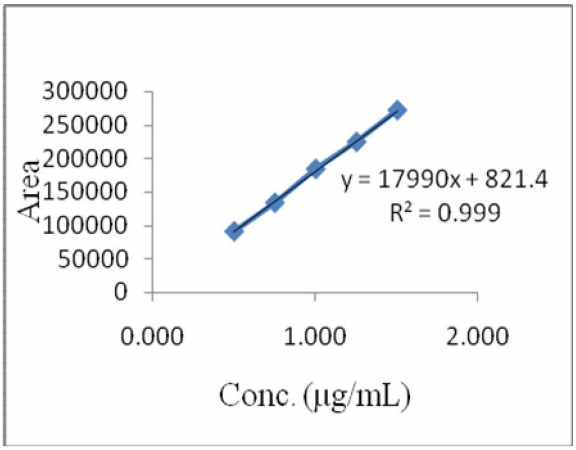


Fig. 1: Chromatogram for Oxcarbazepine and Impurities

Linearity

A good linear relation was observed with the concentration range of 2.5-7.5 µg/mL for Oxcarbazepine with regression equation y=1799906x+821.46(r^2=0.9992) and 0.25-0.75 µg/mL for Impurities with regression equation of Impurity A y=160802x-227.93(r^2=0.9995),Impurity By=150807x-135.82(r^2=0.9994),Impurity C y=264312x+36.036(r^2=0.9995),Impurity D y=886415x-208.44(r^2 =0.9995).Calibration curve were shown in figure 2-6.



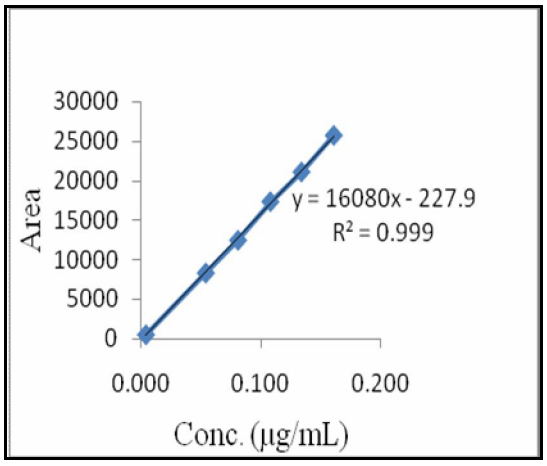
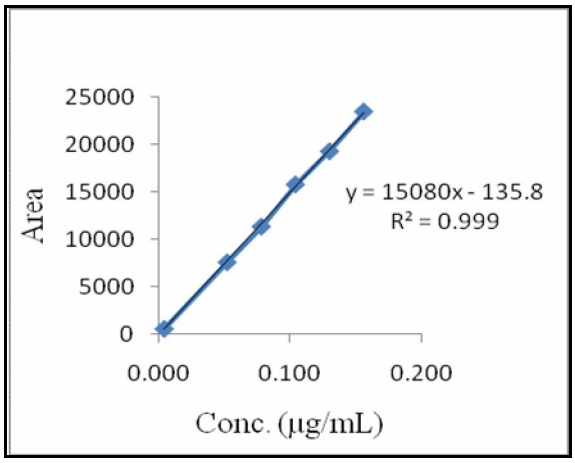


Fig. 2:Calibration curve of OxcarbazepineFig. 3: Calibration curve of ImpurityA



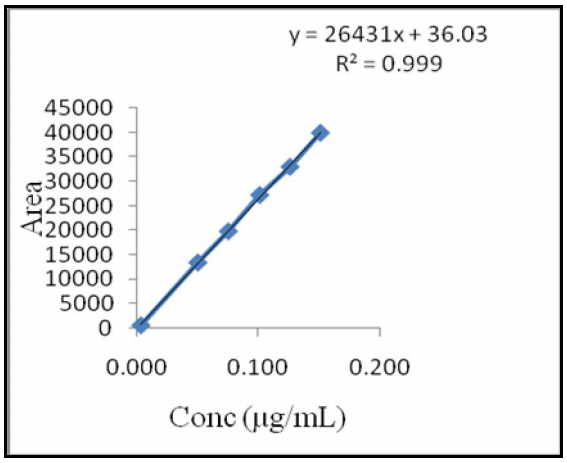


Fig. 4: Calibration curveof Impurity BFig.5: Calibration curve of Impurity C

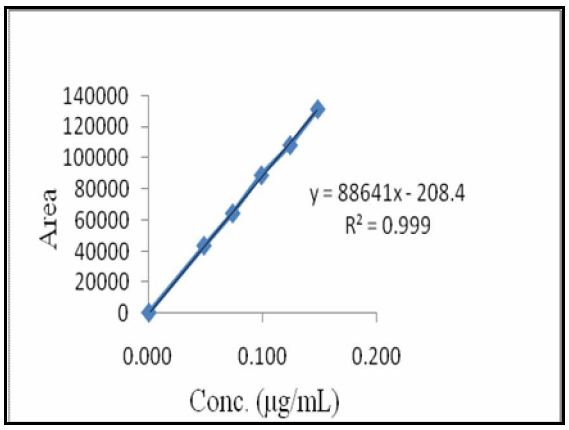


Fig.6:Calibration curve of Impurity D

LOD and LOQ

The limit of detection and limit of quantificationof Oxcarbazepine and its impurities A, B, C and D were shown in Table 2.

Table 2: LOD and LOQ (n=6)

Drug and Impurities	LOD (ng/mL)	LOQ (ng/mL)
Oxcarbazepine	750	1700
Impurity-A	6.0	18.0
Impurity-B	7.3	22.0
Impurity-C	4.3	13.0
Impurity-D	1.0	3.0

Precision:

The precision of the method was expressed as relative standard deviation (RSD, %). The % RSD were found to be less than 2% for Oxcarbazepine and its impurities A, B, C and D, indicating acceptable degree of intra-day and inter-day precision (Table 3 and 4).

Table 3: Intraday precision data

Sample No.	Oxcarbazepine Area	Impurity A Area	Impurity B Area	Impurity C Area	Impurity D Area
1	313128	17253	19424	21519	83963
2	313015	17254	19437	21646	84383
3	312054	17431	19129	22200	84093
4	312965	17349	19401	21562	84198
5	313287	17361	19592	22119	84243
6	301910	17388	19069	21504	84460
Mean	311059.8	17339	19342	21758	84223
SD	4503.28	72.21	200.81	315.68	182.92
% RSD	1.45	0.42	1.04	1.45	0.22

Table 4: Interday precision data

Sample No.	Oxcarbazepine Area	Impurity A Area	Impurity B Area	Impurity C Area	Impurity D Area
1	313828	28691	19787	26348	84409
2	313012	28953	19873	26810	84816
3	301854	28851	19870	26131	85408
4	313465	28457	19893	26491	85029
5	313180	29410	19876	26810	84523
6	311042	28178	20274	26299	84700
Mean	311063.5	28756.67	19928.83	26481.5	84814.17
SD	4615.22	425.0311	173.154	279.2467	363.529
% RSD	1.48	1.48	0.87	1.05	0.43

Accuracy

Accuracy results were expressed as percent recoveries of the particular components in the sample. The overall results of percent recoveries of Oxcarbazepine and its impurities A, B, C and D were indicating good accuracy of the proposed RP-HPLC method. The % recovery was shown in Table 5.

Table 5: Accuracy studies (n=3)

Drug and Impurities	Amount added (%)	Total amount (µg/mL)	Amount recovered (µg/mL)	% Recovery
Oxcarbazepine	80	2	1.98	99.00
	100	2.5	2.49	99.60
	120	3	2.98	99.33
Impurities A	80	0.269	0.267	99.25
	100	0.538	0.535	99.44
	120	0.646	0.641	99.23
Impurities B	80	0.417	0.413	99.04
	100	0.521	0.519	99.62
	120	0.625	0.620	99.20
Impurities C	80	0.402	0.397	98.76
	100	0.503	0.498	99.00
	120	0.604	0.599	99.17
Impurities D	80	0.396	0.394	99.50
	100	0.495	0.490	99.00
	120	0.594	0.589	99.16

Robustness:

Robustness of the method was determined by making slight changes in the experimental conditions such as the wavelength, flow rate and temperature were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstratedthat the RP-HPLC method developed, are robust. The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 6 indicate the robustness of the method.

Table 6: Robustness data

Drug and Impurities	Temperature (°C)		Flow rate (mL/min.)		Wavelength (nm)	
	- 0.5	**+0.5**	**- 0.1**	**+ 0.1**	**- 1**	**+ 1**
Oxcarbazepine	1.12	1.17	1.11	1.13	1.16	1.18
Impurity A	1.07	1.05	1.10	1.15	1.18	1.20
Impurity B	1.13	1.20	1.16	1.12	1.15	1.17
Impurity C	1.18	1.22	1.02	1.08	1.15	1.11
Impurity D	1.09	1.02	1.06	1.04	1.08	1.03

Conclusion

In the present study, the reversed phase HPLC method has been developed for the estimation of Oxcarbazepine and its related substances. It is evident from the study that the method is simple, precise, specific, and accurate. The developed method can be used in the pharmaceutical industry for the routine simultaneous analysis of Oxcarbazepine and its related substances.

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References

1 Satish MA, Nagendrappa G,Spectrophotometric determination of Oxcarbazepine in Pharmaceutical formulations, International journal of pharmacy and pharmaceutical sciences, 2010, 2 (3), 93-98.
2 Paula C RE, Renata B de O, Gerson A P, Oxcarbazepine: Validation and application of an analytical method, Brazilian Journal of Pharmaceutical Sciences, 2010, 46 (2), 265-272.
3 Prameela Rani A, Veesam H, Novel spectrophotometric estimation of oxcarbazepine using mixed hydrotropic technique. International J. Pharmacy and Pharmaceutical sciences, 2012, 4(1), 179-182.
4 Mohan A, Ghosh SK, et.al. , New method for spectrophotometric estimation of new antiepileptic drugs in solid dosage form. Asian Journal of Research in Chemistry, 2009, 2(3), 322-324.
5 Basavaiah K, Rajendraprasad N, Cijo MX, Vinay KB, Ramesh PJ, Development and validation of stability indicating spectrophotometric methods for determination of oxcarbazepine in pharmaceuticals, Journal of Scientific and Industrial Research. 2011, 70, 346-351.
6 Ramaa, CS, Chothe PP, Naik AA, Kadam VJ, Spectrophotometric method for the estimation of oxcarbazepine in tablets. Indian Journal of Pharmaceutical sciences. 2006, 68 (2), 265-266.
7 Rajendraprasad N, Basavaiah K, BasavaiahV K, Volumetric and spectrophotometric determination of oxcarbazepine in tablets. ActaChim. Slov. 2011, 58, 621–628.
8 Bhoite DS, Dhole S N, Bhoir S, Sangole P, Thorat S, Development and validation of stability indicating HPTLC method for determination of Oxcarbazepine in bulk and pharmaceutical formulation. International J. Pharmacy and Pharmaceutical sciences, 2013, 5 (3), 127-132.
9 Suganya S, Bright A, Renuga Devi TS, Studies on linearity and assay using RP-HPLC and UV-Visible spectroscopy for the drugs oxcarbazepine and pioglitazone before and after expiry period. International J. of Pharm Sci and Research. 2012, 3 (6), 1731-1734.

10 Rao KS, Belorkar N, Rao MEB, Development and Validation of Stability-Indicating Liquid Chromatographic Method for the Quantitative Determination of Oxcarbazepine in Tablet Dosage Forms, J Young Pharm, 2009, 1(3), 270-277.
11 Bhaumik U, Bose A, Chatterjee B, Ghosh A, Sengupta P, Agarwal S, Das A, Pal TK, Stability-indicating HPLC method for the determination of oxcarbazepine in pharmaceutical formulation. Asian J. of Chemistry. 2010, 22(3), 2051-2057.
12 Pathare DB, Jadhav AS, Shingare MS, A validated stability indicating LC method for Oxcarbazepine. J Pharm Biomed Anal. 2007, 43(5), 1825-1830.
13 Yang Z, Cui C, Li B, Qiu Z, Feng Y, Lin K, Degradation of carbamazepine and oxcarbazepine by heat-activated persulfate. Acta Scientiae Circumstantiae. 2013, 1, 98-104.
14 Qi ML, Wang P, Wang LJ, Fu RN, LC method for the determination of oxcarbazepine in pharmaceutical preparations. J Pharm Biomed Anal. 2003, 31(1), 57-62.
15 Raghavi K, Sindhura M, Prashanthi R, NalluriBuchi N, Studies on forced degradation of Oxcarbazepine using LC-MS compatible stability indicating RP-HPLC method. Journal of Chemical & Pharmaceutical Research. 2012, 4(8), 3885-3893.
16 Di Corcia D, Lisi S, Pirro V, Gerace E, Salomone A, Vincenti M, Determination of pharmaceutical and illicit drugs in oral fluid by ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B AnalytTechnol Biomed Life Sci, 2013, 927, 133-141.
17 Collins J A, Janis G C, Analysis of selected anticonvulsants by high performance liquid chromatography-tandem mass spectrometry, LC-MS in Drug Analysis, Methods in Molecular Biology, 2012, 902, 201-209.
18 Corso G, D'Apolito O, Paglia G, The accuracy of oxcarbazepine (XC) quantification by a liquid chromatography/tandem mass spectrometry method is influenced by the ion source fragmentation of its metabolite trans-diol-carbazepine (DHD). Rapid Communication in Mass Spectrometry. 2007, 21(2), 269-272.
19 KunduS, GyadangiB, A simple, validated, single HPLC method for the determination of assay, dissolution, related substance of an antiepileptic drug in different pharmaceutical dosage form. Indo American Journal of Pharm Research, 2013, 3(12), 1722-1732.
20 Van Belle K, Verfaillie I, Ebinger G, Michotte Y, Liquid chromatographic assay using a microcolumn coupled to a U-shaped optical cell for high-sensitivity ultraviolet absorbance detection of oxcarbazepine and its major metabolite in microdialysates. J Chromatogr B Biomed Appl., 1995, 672 (1), 97-102.
21 Panda S S, Kumar B Venkata V R, Panda R K, Patel P K, Nagireddy N Rao, Development and validation of a stability-indicating RP-UPLC method for determination of oxcarbazepine in tablet dosage form. Journal of Advanced Pharmaceutical Research. 2012, 3(4), 77- 84.
22 M. EncarnaciónBurgoaCalvo, Olga DomínguezRenedo, M. Julia Arcos Martínez, Determination of oxcarbazepine by Square Wave Adsorptive Stripping Voltammetry in pharmaceutical preparations. J. Pharm Biomed Anal. 2007, 43(3), 1156-1160.
23 Dominguez-Renedo Olga, CalvoEncarnacionBurgoa M, Alonso-Lomillo Asuncion M, Arcos-Martinez Julia J, Oxcarbazepine analysis by adsorptive stripping voltammetry using silver nanoparticle-modified carbon screen-printed electrodes. Sensor Letters. 2010, 8(2), 268-272.
24 ICH, Validation of Analytical Procedure: Text Methodology Q2 (R1), US Food and drug Administration, 2005.

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