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Spectrophotometric Determination of Certain Antiepileptic's in Tablets Using Vanillin Reagent

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ABSTRACT

A selective and new spectrophotometric method is described for determination of three antiepileptic drugs; namely lamotrigine (LAM), gabapentin (GAB), and oxcarbazepine (OXC) in drug substances and in drug products using vanillin reagent as the chromogenic agent.The method is based on a coupling reaction between the cited drugs and vanillin reagent in acidic condition. Under optimized conditions, the yellow colored products were measured at 405, 396, and 400 nm respectively. Beer's law was obeyed at (0.4 – 10), (0.1-10), and (0.5-11) µg/mL, and the calculated molar absorptivity values are 2.52 x 10^4, 1.74 x 10^4, and 2.54 x 10^4 L/mol/cm for LAM, GAB, and OXC respectively. Sandell sensitivity, the limit of detection (LOD) and limit of quantification (LOQ) were calculated. No interference was observed from common additives found in drug products. The presented method was validated according to ICH guidelines. Statistical comparison of the results was performed using Student's *t*-test and *F*-ratio at 95% confidence level, and there was no significant difference between the reference and proposed method with regard to accuracy and precision. The method offers the advantages of rapidity, simplicity and sensitivity and low cost and can be easily applied to resource poor settings without the need for expensive instrumentation and reagents.

Keywords: Antiepileptic drugs; Chromogenic agent; ICH guidelines; Statistical comparison; Spectrophotometric method; Vanillin reagent.

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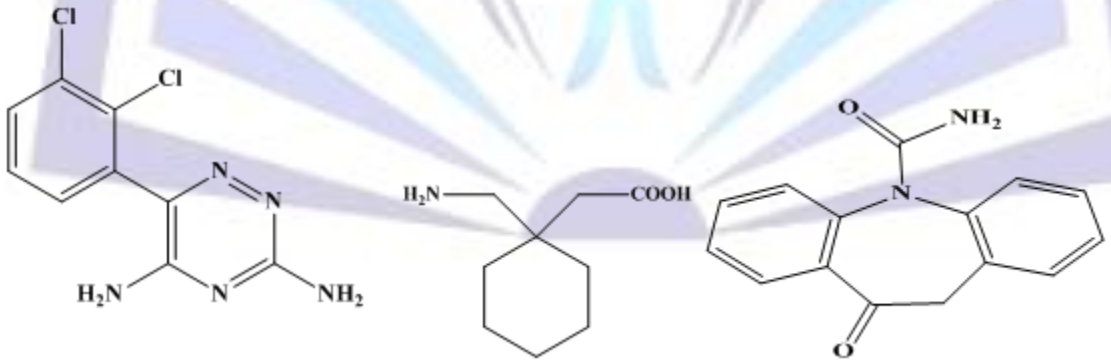
1. INTRODUCTION:

Lamotrigine (LAM), 3, 5-diamino-6-(2, 3-dichlorophenyl)-1, 2, 4-triazine Figure 1is an anticonvulsant drug. As an antiepileptic, it has been used successfully to treat epilepsy and bipolar disorder as immunotherapy and as an adjunct with other antiepileptic for treatment of partial and generalized toxic-chronic seizures. It is also used to treat neurological lesions and as a tranquilizer [1, 2]. LAM is the subject of monographs in the United States Pharmacopeia[3] where by potentiometric method is recommended for its determination. Several analytical methods have been reported for the determination of LAM in pharmaceuticals or in biological fluids including reverse-phase HPLC [4-6], GC with nitrogen phosphorous detector[7], capillary electrophoresis[8], adsorptive stripping voltammetry[9,10] and spectrophotometry[11-17]. The objective of the present work is to develop simple, rapid and precise UV spectroscopic method for the determination of lamotrigine in drug substance and in drug products.

The new anti-convulsion drug Gabapentin (1-(amino methyl) cyclohexane acetic acid) Figure 1 is a structural analogue of amino butyric acid (GABA) and its action is attributed to the irreversible inhibition of the enzyme GABA-transaminase, thus preventing the physiological degradation of GABA in the brain. [18] Currently, GAB and its pharmaceutical dosage forms are official in the United States Pharmacopeia and also different analytical methods are reported for its determination. These include high performance liquid chromatography [3], liquid chromatography-mass spectrometry [19], gas chromatography mass spectrometry [20], capillary electrophoresis [21], potentiometric [22], spectrofluorimetric [23], and colorimetric. [24-27]. Literature survey does not reveal any simple extractive spectroscopic method for determination of GBP. The present manuscript describes simple and sensitive spectroscopic procedures for the determination of GAB in drug products.

Oxcarbazepine (10, 11-dihydro-10-oxo-5H-dibenzo [b, f] aze-pine-5-carboxamide), Figure 1.An antiepileptic drug, official in the United States Pharmacopeia [3] is a 10-keto analogue of carbamazepine with a similar therapeutic profile, but with less adverse effects and less clinical relevant pharmacokinetic drug interactions [28]. OXC is indicated as first line drug in immunotherapy or polytherapy for the treatment of partial seizures with or without secondarily generalized tonic colonic epileptic seizures [29-30]. These actions are thought to be important in the prevention of synaptic neurotransmission and seizure spread in the intact brain [31]. In addition,increased potassium conductance and modulation of high voltage activated calcium channels may contribute to the anticonvulsant effects of the drug [32]. There is LC, GC, voltammetry, HPLCand several other methods for the quantification of oxcarbazepine and its main metabolites 10-hydroxy-10, 11- dihydrocarbamazepine and 10, 11- dihydroxy-trans-10, 11- dihydrocarbamazepine in biological fluids, which are reported [33-40], another method spectrophotometry[41-42] and voltammetry[43]. To our knowledge most of the reported methods mainly describe the determination of OXC in biological fluids but only a few methods are describing its determination in pharmaceutical formulations. In addition some of those methods are requiring expensive equipment, reagents and are also time consuming; an attempt is made here to develop a simple, rapid, economical and sensitive spectrophotometric method based on the reaction of OXC with vanillin in acidic medium for its determination either in pure or in dosage form.

Vanillin (4-hydroxy-3- methoxybenzaldehyde) Figure 1 is an organic compound containing an aldehyde, ether and phenolic moiety. It has been used as a chemical intermediate in the production of pharmaceuticals and other chemicals. It is also used as a general purpose stain for TLC plate development to aid visualization. Use of it has also been reported in the quantitative determination of certain drugs by spectrophotometric reaction [44-49].



(a) (b) (c)

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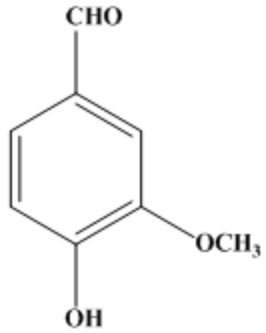


(d)

Figure 1:Chemical structures of the investigated antiepileptic drugs (a) lamotrigine, (b) gabapentin, (c) oxcarbazepine and (d) vanillin reagent.

2. EXPERIMENTAL PROCEDURES:

2.1. Instrument

SHIMADZU UV-2450 PC Series Spectrophotometer (Tokyo – Japan) with two matched 1 cm quartz cells using the following spectral parameters: Scan mode: absorbance, Speed: fast and Slit width: 2 nm

2.2. Materials and Chemicals

Lamotrigine (Batch no: 14LM000002) was kindly supplied by Delta Pharm and assayed for purity according to the official HPLC assay [3] to contain 99.95%±0.12%. Larogen 100 mg/tab. (B.N. 13691), manufactured by Delta Pharm. Gabapentin (Batch no: 20121004) was kindly supplied by Mash Pharm. industry and assayed for purity according to the official HPLC assay [3] to contain 98.85%±0.23%. Gaptin 100 mg/tab. (B.N. GA102) manufactured by Delta Pharm. Oxcarbazepine (Batch no: OX0010514) was kindly supplied by Mash Pharm pharmaceutical industry and assayed for purity according to the official HPLC assay [3] to contain 99.87%±0.28%. Trileptal 150 mg/tab. (B.N. 10000), manufactured by Novartis Com.

All chemicals are analytical grad, Methanol (LAB-SCAN), Hydrochloric acid 37% (Honeywell), and Vanillin (Qualikems Fine Chemicals Pvt. Lid), Prepared by dissolving 5 gm. of vanillin in 100.0 mL of 1.0 M methanolic hydrochloric acid.

2.3. Standard Stock Solution of Drugs

Accurately about 20 mg of the drug substances (LAM, GAB, OXC) was weighed and dissolved in 1.0 M methanolic hydrochloric acid and the volume is made up to the volume 100 ml to give standard stock solution (200µg/ml) and from this solution 10 ml of sample was transferred in to separated 100 ml volumetric flask and volume was made up to the mark 100 ml with 1.0 M methanolic hydrochloric acid to get concentration (20µg/ml) as a working stander solution.

2.4. Procedure for Calibration Curves

An aliquot of 1.0 mL of the working stander solutions was transferred into a 10-mL calibrated flask, 2.0 mL of 5% vanillin reagent was added, mixed well, the reaction was allowed to stand for about15 minutes, then the resulting colored products were measured at 405, 396, and 400 nm for LAM, GAB, and OXC respectively against blanks which treated similarly.

2.5. Procedure for the Assay of Drug Products

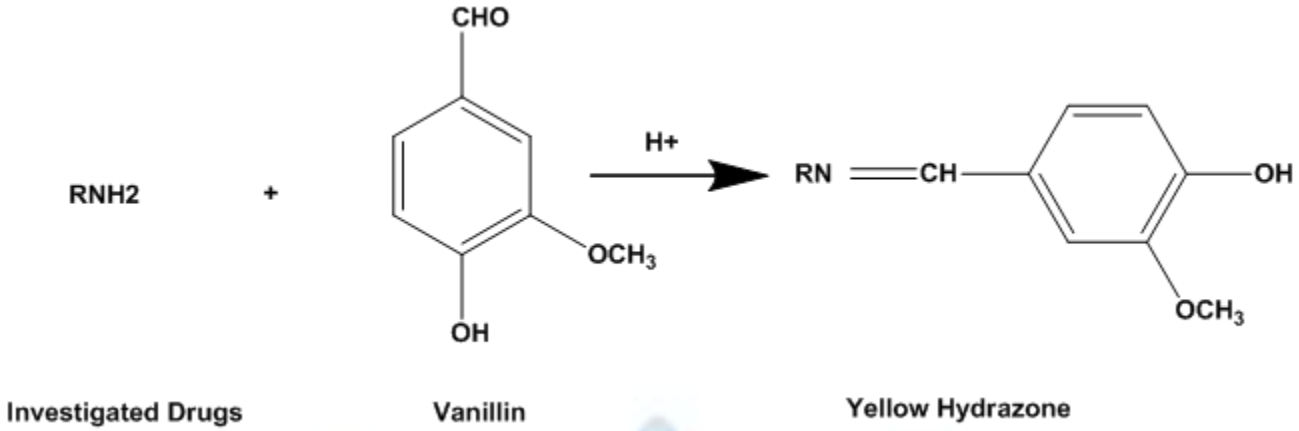
Twenty tablets were weighted accurately, the contents were mixed thoroughly.An accurate weight of the mixed powder equivalent to one tablet of LAM, GAB, and OXC were transferred into three separated 100-ml conical flasks, 50-ml 1M methanolic hydrochloric acid was added, The contents were swirled and sonicated for 10 min, then filtered through a What- mann No. 42 filter paper previously moisten with methanol. The collected filtrate was transferred quantitatively into 100-mL calibrated flask; the resultant solutions were completed to mark with 1.0 M methanolic hydrochloric acid and then subjected to subsequent dilution.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

LAM, GAB, and OXC readily reacts with vanillin in methanolic hydrochloric acid solution yielding an intense yellow colored hydrazone as shown in the equation having an absorption maximum at 405,396, and400 nm (Schema 1). The reagent blank does not absorb around this wavelength. The hydrazone formed was stable in the temperature range

20 – 40^{0}C. The color of the products formed was stable for about 2 hours at room temperature (30^{0}C) followed by a steady decrease in absorbance values as shown in Figure 2.



Scheme 1: Probable reaction scheme.

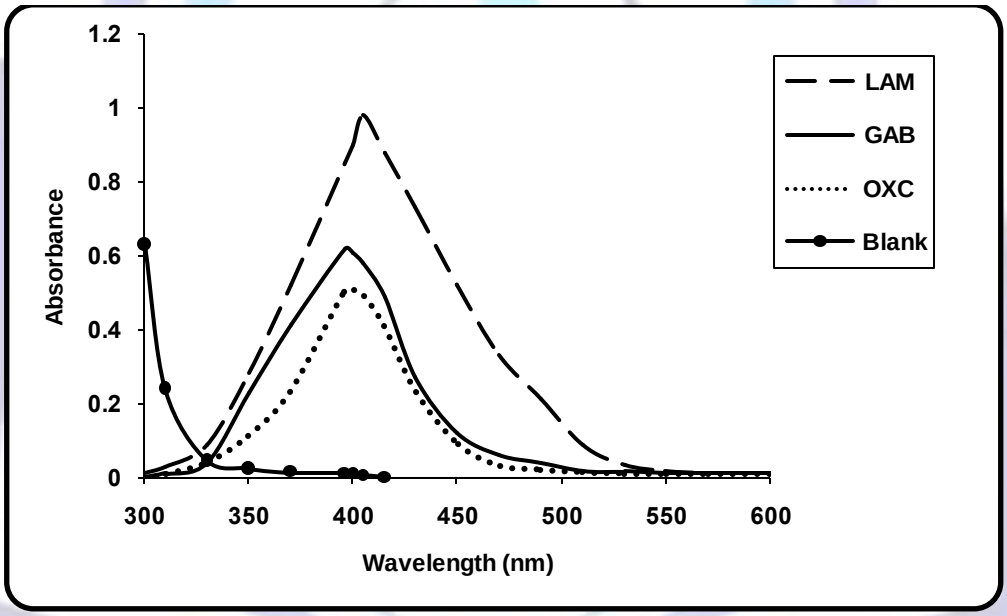


Figure 2:Absorption spectra of LAM (10 µgml^{-1}), GAB (6µgml^{-1}), and OXC (5µgml^{-1}) - Vanillin (5 %w/v) reaction product.

3.2. Optimization of Experimental Variables

Various experimental variables were optimized to achieve maximum sensitivity.

3.2.1. Determination of effective reagent concentration

Preliminary experiments were done in order to ascertain the effect of concentration and the volume of vanillin at the wavelength of maximum absorption, 405,396, and 400nm. To series of LAM, GAB, and OXC solutions, varying concentrations **(**1-7%) were added and the analytical procedure followed. After 15 minutes, the absorbance of each solution of three drugs was read at 405,396, and 400nm. It was observed that the analytical signal increased with an increase in reagent concentration up to 6%. The concentration of vanillin therefore utilized was 5%. Similarly, by fixing the vanillin concentration as 5% in series of LAM, GAB, and OXC solutions, different volumes of vanillin in the range of 0.5 – 4 ml were added. The analytical procedure was then followed, after 15 minutes, the absorbance was read. It was observed that 2-ml of 5% vanillin solution was optimal for the formation of color with maximum intensity. Therefore, 2-ml of 5% vanillin solution was utilized for all measurements as shown inFigure 3.

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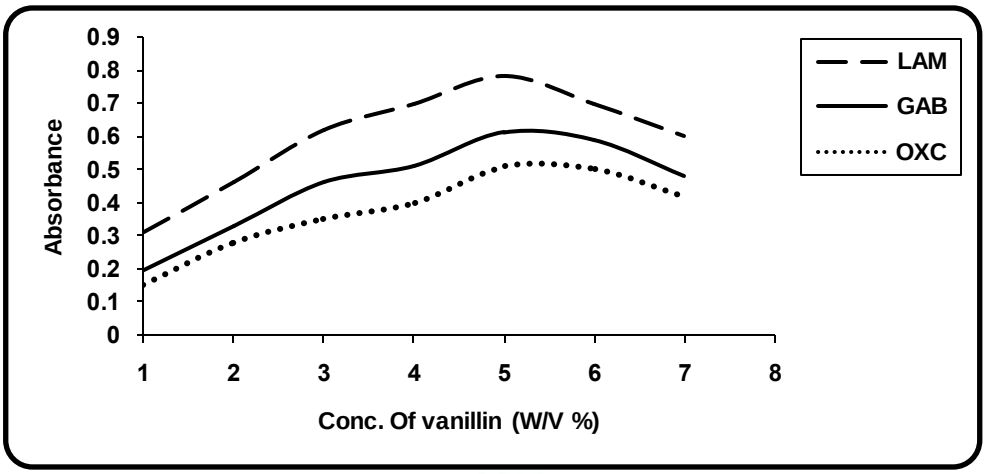

Figure 3:Effect of conc. of (2-ml) of Vanillin in absorbance of LAM (10 µgml^{-1}), GAB (6µgml^{-1}), and OXC (5µgml^{-1}) – Vanillin (5% w/v) reaction products.

3.2.2. Types of acids

Reaction between vanillin reagent and antiepileptic drugs was found to proceed in acidic medium. So, different acids were tested. 1.0 M methanolic Hydrochloric acid resulted in an increase of the absorbance intensity accompanied by hyper chromic shift as shown inFigure 4.

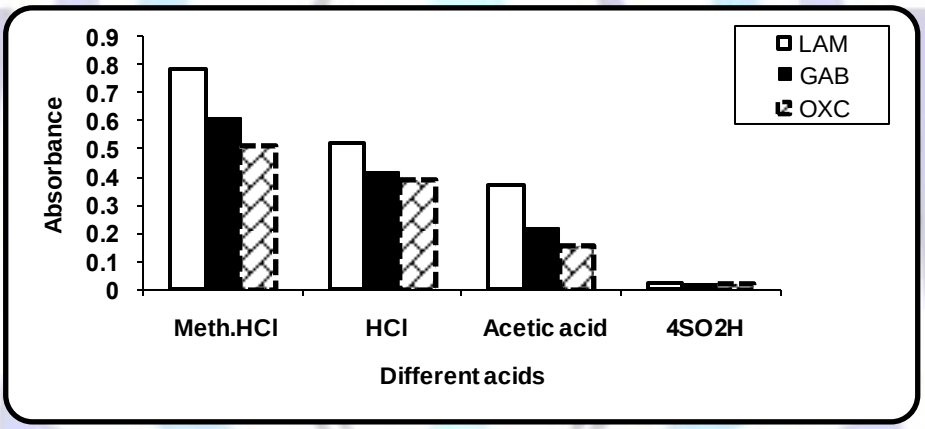

Figure 4: Effect of different acids on absorbance of LAM (10 µgml^{-1}), GAB (6µgml^{-1}), and OXC (5µgml^{-1}) –Vanillin (5% w/v) reaction products.

3.2.3. Effect of reaction time

The effect of time on the reaction was studied by carrying out the reaction for different periods of time (0-30 min). The reaction product's absorbance of LAM, GAB, and OXC with vanillin wasincreased by increasing the time up to 20min. The color of the products formed was stable for about 2 hours at room temperature (30 ^{0}C) followed by a steady decrease in absorbance values as shown in Figure 5.

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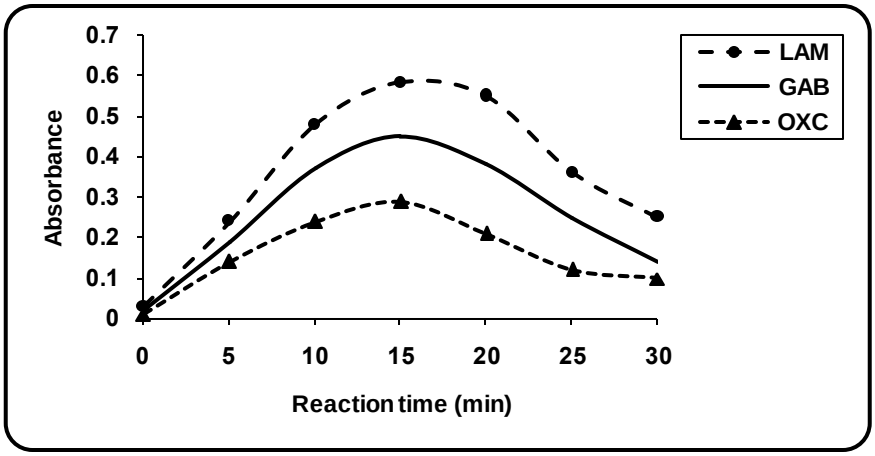

Figure 5: Effect of reaction time on absorbance of LAM (6 µgml^{-1}), GAB (4µgml^{-1}), and OXC (3µgml^{-1}) –Vanillin (5% w/v) reaction products.

3.2.4. Stability of the chromophore

After dilution the reaction solutions, it was found that the absorbance of the chromophore (LAM-vanillin), (GAB-vanillin), and (OXC-vanillin) remained stable for at least 2 hours. Under the optimum experimental conditions the calibration curves were plotted representing the relationship between the absorbance at 405, 396 and 400 nm and the corresponding concentration of the three cited drugs (LAM, GAB, and OXC). Linear correlation coefficients were obtained within the concentration range (0.4 – 10), (0.1-10), and (0.5-11) µg/mL for LAM, GAB, and OXC respectively.

3.2.5. Stoichiometry of the reaction

Under the optimum conditions, the stoichiometry of the reaction of LAM, GAB, and OXC with vanillin were determined adopting the Job's method of continuous variation [50]. The results revealed that LAM, GAB, and OXA reacted with vanillin in a ratio of 1:1 under the optimum condition attained Figure 6.

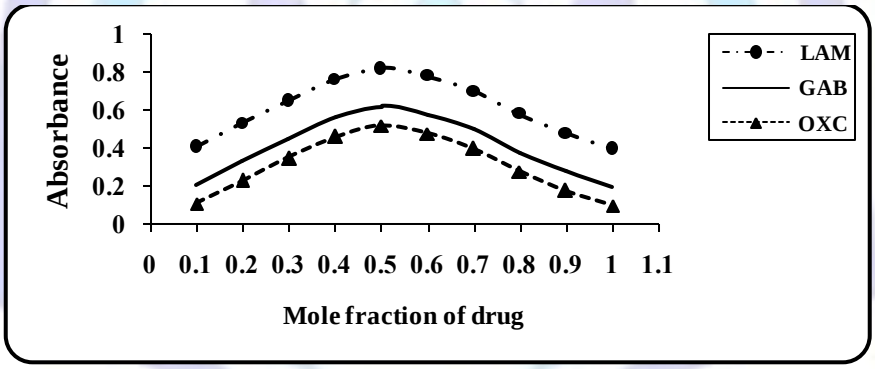

Figure 6 :Determination of Stoichiometry of the reaction of LAM, GAB, and OXC– vanillin (5% w/v) by Continuous Variation method using (2 x 10^{-4} M) solutions.

3.2.6. Determination of the stability constant

The stability constant (K_f) of the reaction products are calculated according to the follow equation:

$K_f = (A / A_m) / [1 - A / A_m]\, n^{-1} C^n n^n$ [51]

Where: A = maximum absorbance of the continuous variation curve Figure 6, A_m = absorbance corresponding to intersection of two tangents of the continuous variation curve, n = number of molecules of the reagent in the reaction product, C = molar concentration of the drug and K_f= formation constant of the complex.

The stability constant of the reaction products of LAM, GAB and OXC with vanillin were 4.2 x 10^4, 1.8 x 10^4, and 2.4 x 10^3, respectively.

The Gibbs free energy change of the reaction (ΔG) [52] was also calculated adopting the following equation: $\Delta G = - 2.303\, R\, T \log K_f$

Where: ΔG = Gibbs free energy change of the reaction (k.J. mol^{-1})

R = Universal gas constant (8.314 joules)

T = Absolute temperature (273+25^{o}C)

K_f = Formation constant of reaction

The free energy changes (ΔG) of the reaction LAM, GAB and OXC with vanillin was found to be – 6.3 x 10^4, - 5.8 x 10^4 and – 4.6 x 10^4 k.J.mole^{-1} respectively, The higher K_f and ΔG values obtained indicate very stable reaction products.

3.3. Method Validation

3.3.1. Analytical Parameters

A linear relation was found to exist between absorbance and the concentration of LAM, GAB, and OXC in the range (0.4 – 10), (0.1-10), and (0.5-11) µg/mLrespectively. The calibration graph is described by the equation: Y = a+bC (where Y is the absorbance, a is the intercept, b is the slope and C is the concentration in µg/mL) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection and quantification calculated as per the current ICH Q2B guidelines [53] are compiled in Table 1 and are indicative of the excellent sensitivity of the method. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae: LOD = 3.3σ /b and LOQ = 10 σ /b, where b is slope of the calibration curve and σ is standard deviation of y-intercept of regression equation Table 1.

Table 1:Sensitivity and regression parameters

Parameters	Lamotrigine	Gabapentin	Oxcarbazepine
λ max	405.0nm	396.0 nm	400.0 nm
Molar absorptivity (L mol^{-1} cm^{-1})	2.52x10^4	1.74x 10^3	2.54x10^4
Sandell sensitivity (µcm^{-2} /0.001 abs unit)	10.23x10^{-3}	9.71x10^{-3}	9.82x10^{-3}
Regression equation (Y= bC+ a)			
Slope (b)	0.0985	0.1017	0.1007
SD of slope	0.000135	0.00083	0.00195
S E of slope	0.000555	0.00034	0.00079
Confidence limit of slope	0.09718 – 0.09989	0.10083 – 0.10249	0.09866 – 0.10275
Intercept (a)	- 0.001	0.0034	0.0043
SD of Intercept	0.007153	0.004378	0.0125
S E of Intercept	0.002921	0.001788	0.00508
Confidence limit of Intercept	-0.00815 – 0.00615	-0.00095-0.0078	-0.00173 –0.01738
Correlation coefficient (r)	0.9998	0.9999	0.9997
SE of (r)	0.00533	0.00334	0.00779
Validation parameters			
Selectivity and Specificity (t-test) (2.228)	0.175	1.15	0.491
Accuracy (Mean^{c} ± RSD %)	99.845 ± 0.867 %	99.0 ± 0.957 %	99.916 ± 0.862%
Linearity (µg /ml)	0.4 -10.0 µg /ml	0.1 -10.0 µg /ml	0.5 -11.0 µg /ml
Limit of detection [LOD]	0.165 µg /ml	0.049 µg /ml	0.188 µg /ml
Limit of quantitation [LOQ]	0.500 µg /ml	0.150 µg /ml	0.570 µg /ml
Robustness(mean% recovery ±S.D)	100.11% ± 0.545	100.08% ± 0.416	100.4% ± 0.583

Y = bC + a where C is the concentration of three drugs in µg /ml and Y is absorbance unit. (Each value is a result of six separate determinations), c 95% confidence limit.

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3.3.2. Accuracy

The accuracy of the proposed method for drug substances and drug products was determined by investigating the percentage recovery at five levels each three times in the concentration range 3.0 -11.0µg/ml as shown in Table 2. The percentage relative standard deviation (% RSD) revealed high accuracy.

Table 2: Accuracy of the proposed method in drug substances and drug products

Conc. taken µg /ml	Lamotrigine		Gabapentin		Oxcarbazepine	
	Drug subs.	Drug product Laroge 100 mg/tab	Drug subs.	Drug product Gaptin 100 mg/tab.	Drug subs.	Drug product Trileptal 150 mg/tab.
	Recovery (%)[a]		Recovery (%)[a]		Recovery (%)[a]	
3	99.23	99.51	98.33	99.67	99.52	99.11
5	99.56	98.72	98.91	98.31	98.92	98.64
7	98.21	99.66	98.73	97.99	100.02	99.09
9	97.75	98.90	99.64	99.12	101.0	99.82
11	100.0	98.81	101.0	99.25	99.76	98.76
Mean± RSD%	98.95 ± 0.951	99.12 ± 0.436	99.32 ± 1.05	98.87 ± 0.703	99.84± 0.765	99.08 ± 0.463

[a]Average from four different experiments. For drug product, recovery is given as a percentage of the amount claimed table.

3.3.3. Precision

The intraday precision was evaluated by assaying freshly prepared solutions in triplicate in the concentration range 2.0 -16.0µg/ml as shown in Table 3. The percentage relative standard deviations (%RSD) were calculated. While interday precision was calculated by assaying freshly prepared solutions in triplicate for three days. The percentage relative standard deviations (%RSD) were calculated as shown in Table 3.

Table 3: Evaluation of intra-day and inter-day of precision

Drug taken µg /ml	Lamotrigine				Gabapentin				Oxcarbazepine			
	Intra-day accuracy and precision		Inter-day accuracy and precision		Intra-day accuracy and precision		Inter-day accuracy and precision		Intra-day accuracy and precision		Inter-day accuracy and precision	
	Lam. found	RSD%	Lam. found	RSD%	Gab. found	RSD%	Gab. found	RSD%	Oxc. found	RSD%	Oxc. found	RSD%
4	4.02	0.123	3.90	0.505	4.00	0.214	3.89	0.641	4.06	0.136	3.95	0.321
6	6.10	0.265	6.01	0.541	6.05	0.341	5.97	0.521	6.03	0.710	5.94	0.221
8	8.01	0.348	8.04	0.364	7.90	0.721	7.99	0.543	8.01	0.213	8.00	0.334

RSD%: relative standard deviation.

3.3.4. Robustness and ruggedness

Method robustness was tested by making small incremental changes in volume of vanillin rang of Temp. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst. The robustness and the ruggedness were checked at three different drugs levels. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the Table 4.

Table 4: Robustness and ruggedness expressed as intermediate precision (% RSD)

Drugs	Method Robustness		Method Ruggedness	
	Parameters altered			
	Vanillina (5%) ml, RSD % (n=3)	Change in Tempb.(30oC) RSD % (n=3)	Inter-analysts' RSD % (n=3)	Inter-instruments' RSD % (n= 3)
Lamotrigine	0.755%	0.252%	0.261%	0.448%
Gabapentin	0.843%	0.836%	0.684%	0.297%
Oxcarbazepine	0.794%	0.504%	0.107%	0.905

a= vanillin volumes were used 2.1, 2.3, 2.5 ml.

b= change in Temp. 30.2, 30.4, 30.6 ^{o}C.

3.3.5. Method Validation for Drug Products

It is evident from the above-mentioned results that the proposed method gave satisfactory results with LAM, GAB, and OXC in its drug substances. Thus its drug products were subjected to the analysis of their LAM, GAB, and OXC contents by the proposed and the potentiometric method for LAM (4), official HPLC method for GAB (4) and official HPLC method for OXC (28). The label claim percentages were 99.12 ± 0.436%, 99.32 ± 1.05%, and 99.08 ± 0.463% for LAM, GAB, and OXC, respectively Table 5. These results were compared with those obtained from the official methods by statistical analysis with respect to the accuracy (by *t- test*) and precision (by *F- test*). No significant differenceswere found between the calculated and theoretical values of *t-test* and *F-test* at 95% confidence level proving similar accuracy and precision in the determination of LAM, GAB and OXC by UV method.

Table 5: Results of analysis of tablets by the proposed method

	Lamotrigine *Larogen* *100 mg/tab*		Gabapentin *Gaptin* *100 mg/tab.*		Oxcarbazepine *Trileptal* *150 mg/tab.*	
	proposed method	HPLC method [3]	proposed method	HPLC method [3]	Proposed method	HPLC method [3]
Mean* ***± RSD%***	99.57± 0.483%	99.53± 0.49%	99.77± 0.216%	99.65± 0.182%	99.61± 0.272%	99.54± 0.253%
Variance	0.231	0.238	0.046	0.033	0.073	0.064
SE	0.196	0.199	0.086	0.074	0.111	0.103
t-test (2.228)[a]	0.144		0.902		0.461	
F-test (5.1)[a]	1.030		1.39		1.14	

Average of six different experiments a) Theoretical values

4. CONCLUSION

A simple, sensitive and rapid method for the determination of LAM, GAB and OXC in its drug products are described, involving the use of vanillin as a chromogenic agent. The method is rapid and less tedious than many reported spectrophotometric methods. The present method can be applied at ambient temperature; color development is instantaneous and does not require strict pH control or tedious liquid-liquid extraction step. The method employs inexpensive and easily available chemicals and instrument. As most relevant features of the method, high sensitivity and a wide linear dynamic range compared to many existing methods can be emphasized. The color formed is highly stable leading to very precise results. These advantages make the method a valuable alternative to many existing methods for the determination of LAM, GAB and OXC in drug substances and can be applied in biological fluids.

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