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METHOD DEVELOPMENT AND VALIDATION OF PREGABALIN AND ETORICOXIB IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Abstract:

A simple, accurate, precise and highly selective revers phase high performance liquid chromatography (RP-HPLC) method was developed and velaidated for pregabalin and etoricoxib. Chromatographic separation was achived isocratically by using Thermo scientific Accela Autosampler HPLC system, C8 column (250mm × 4.6 mm I, d.5µ). flow rate selected was 1ml/min. Detection wavelength 210 nm and sample volume 10µl , run time 10 min. the mobile phase employed was potassium dihydrogen orthophosphate buffer pH 6 - with Ortho phosphoric acid : Methanol : Acetonitrile (70:15:15 % V/V/V) which resulted best resolution and sensitivity. Developed method was validated in terms of linearity range (from 120-195 µg/ml of Pregabalin and 96-156 µg/ml of Etoricoxib) correlation coefficient was found for 0.9996 of PRE and 0.9998 ETO. The low RSD values indicate good precision and high recovery values indicate accuracy of the proposed method. The proposed method has been applied to the determination of drugs in commercial formulations. Assay results were in good agreement with label claim was found to be 99.56% and 100.16% of Pregabalin and Etoricoxib. The method was validated as per ICH guidelines. The developed method was simple, accurate, precise, specific, sensitive and reproducible which can be efficiently and easily applied to pharmaceutical dosage forms.

KEYWORDS: *Pregabalin, Etoricoxib, HPLC, potassium dihydrogen orthophosphate buffer.*

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INTRODUCTION :

Pregabalin (PRE) is chemically (3S)-3-(aminomethyl)-5-methylhexanoic acid (Fig.1). The structure of Pregabalin is similar to Gamma-aminobutyric acid (GABA), it does not bind to GABA receptors. Instead, it binds the alpha2-delta subunit of presynaptic voltage-gated calcium channels in the central nervous system. Pregabalin does not modulate dopamine receptors, serotonin receptors, opiate receptors, sodium channels or cyclooxygenase activity. Etoricoxib (ETO) 5-chloro-3-(4-methanesulfonylphenyl)-2-(6-methylpyridin-3-yl) pyridine (Fig.2) selective COX-2 inhibitor ("coxib"), etoricoxib selectively inhibits isoform 2 of the enzyme cyclooxygenase (COX-2). It has approximately 106-fold selectivity for COX-2 inhibition over COX-1. This reduces the generation of prostaglandins (PGs) from arachidonic acid. Among the different functions exerted by PGs, their role in the inflammation cascade should be highlighted. This both drug is used in the treatment of neuropathic pain.

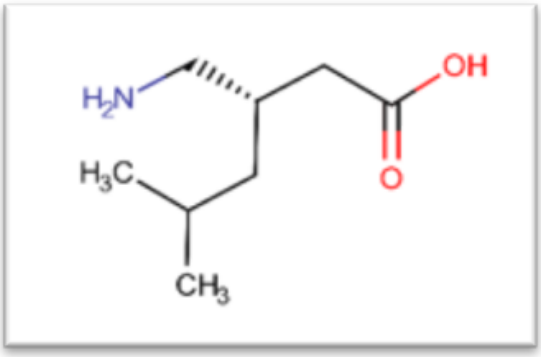

Fig. 1: Structure of pregabalin.

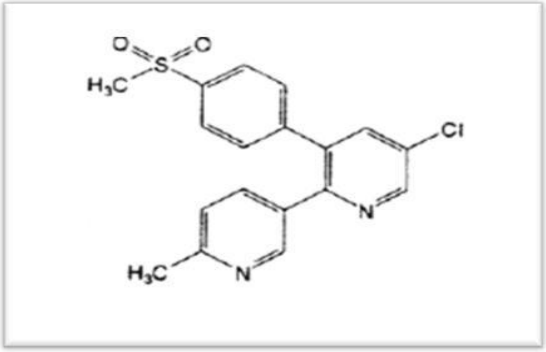

Fig. 2: Structure of etoricoxib.

Literature survey revealed that there are some methods reported for the simultaneous estimation of these drugs, some methods for estimation of individual drugs[7-9] or with other drugs,[10-11] UV-spectrophotometry,[12-14] RP-HPLC.[15-18] There is no method of estimation by RP-HPLC method available in the literature for Simultaneous estimation of Pregabalin and Etoricoxib .So we have decided to develop and validate a new simple, rapid, accurate, specific and highly sensitive and economical simultaneous estimation of RP-HPLC method for Pregabalin and Etoricoxib. The developed method was validated as per ICH norms. [19-20]

MATERIALS AND METHODS:

Instrumentation

The instrument used in the present study was Thermo scientific Accela Autosampler HPLC system. All weighing was done on electronic balance (Model Shimadzu AUX -220).

Reagents and Chemicals

Analytically pure sample of PRE, ETO was kindly supplied by "Ocean pharmaceutical" (Chennai, India). The pharmaceutical dosage form used in this study was a Etoshine-NP tablets manufactured by Sun Pharmaceutical industries Ltd (Haryana, India) labeled to contain 75mg of Pregabalin, 60 mg of Etoricoxib.

Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Water (HPLC Grade), Potassium dihydrogen ortho phosphate buffer (HPLC grade)

Preparation of Buffer Solution:

Weighed accurately 1.36g of potassium dihydrogen ortho phosphate and dissolved 500 ml of water and added for 1ml of Tri ethyl amine and adjusted to pH 6 with ortho phosphoric acid.

Selection of mobile phase and λ max

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this potassium dihydrogen orthophosphate buffer pH 6 - with Ortho phosphoric acid : Methanol : Acetonitrile (70:15:15 % V/V/V) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

The detection wavelength was measured by scanning the solution of Pregabalin and Etoricoxib in mobile phase, in UV- spectrophotometry, overlaid spectra and the wavelength of maximum absorption was selected as 210 nm.

Optimized Chromatographic Conditions

The following parameters were used for RP-HPLC analysis of Pregabalin and Etoricoxib.

Mode of operation - Isocratic

Stationary phase - C8 column (250mm × 4.6 mm I, d.5µ)
Mobile phase - Potassium dihydrogen orthophosphate pH -6: Methanol: ACN (70:15:15) v/v.
Detection wavelength - 210 nm
Flow rate - 1 ml / min
Temperature - Ambient
Sample volume - 10 µl

Preparation of the standard stock solution
Standard Pregabalin stock solution
Weighed accurately 75mg Pregabalin and transferred into a 50 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC grade methanol and the volume was made up to the mark with HPLC grade methanol to get the concentration of 1500 µg/ml for Pregabalin.

Standard Etoricoxib stock solution
Weighed accurately 60 mg of Etoricoxib and transferred into a 50 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC grade methanol and the volume was made up to the mark with HPLC grade methanol to get the concentration of 1200 µg/ml for Etoricoxib
Linearity and calibration:
From the standard solution, pipetted out 4-6.5 ml into a series of six 50 ml volumetric flask and made up to the mark with mobile phase to obtain the concentration range from 120-195 µg/ml of Pregabalin and 96-156 µg/ml of Etoricoxib solution were injected and chromatogram was recorded. The calibration curve was plotted between concentration verses peak area.

Quantification of Pregabalin and Etoricoxib
Twenty Tablets containing Pregabalin 75 mg and 60 mg of Etoricoxib of formulation (Etoshine – NP) accurately weighed. Weighed the content of drug equivalent to 75 mg of Pregabalin was transferred to a 50 ml volumetric flask and dissolved in HPLC grade methanol and sonicated for 15 minutes. The final concentration was 1500 µg/ml. The above solution was filtered through whatmann filter paper No 41 and the clear solution was collected, 5 ml was pipetted into a 50 ml volumetric flask and made up to the mark with the methanol to produce 150 µg/ml solutions. The peak area measurements were done by injecting sample six times and the amount of Pregabalin and Etoricoxib concentrations were calculated from their respective calibration curve.

Recovery Studies
The recovery studies were done by adding known concentration of raw material Pregabalin and Etoricoxib to pre-analysed formulation. The tablet powder equivalent to 75mg of Pregabalin was weighed and transferred into a series of 50ml standard flask (two sets 3 flask). To that 7.5mg, 15mg and 22.5mg of raw material for Pregabalin (one set 3 flask) and 6mg, 12mg and 18mg of Etoricoxib were added (110%, 120%, 130%) and dissolved with diluent. Then it was made up to the volume with the same. Then the solutions were sonicated for 15 minutes. After, sonication the solutions were filtered through Whatmann filter paper No 41 separately. From the clear solution, 5ml of each test solution was pipetted out and made into six replicates for 50ml volumetric flask. 10 µl solutions of each concentration were injected and the chromatograms were recorded. Each concentration was repeated for three times.

Limit of Detection and Limit of Quantification:
Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification of each were calculated by using the average value of slope and standard deviation of response (Intercept).

System Suitability Studies
The system suitability studies were carried out as specified in I.P. the parameter like Column efficiency, Tailing factor, Asymmetric factor, and Theoretical plate number and were calculated.

RESULTS AND DISCUSSION:
An involvement was made in this project to device, a simple, accurate, less expensive and sensitive RP-HPLC method of estimation of Pregabalin and Etoricoxib in solid dosage form. Since the drug is polar reverse phase high performance liquid chromatography was selected.

Selection of Mobile Phase
Acetonitrile was preferred because of its lower viscosity and high UV transparency. Methanol was selected due to its inexpensiveness and also it is a good diluent for this drug. Acetonitrile: Methanol: Water in different ratio was tried and elution of was founded as splitted. Hence choice of orthophosphoric acid was incorporated to adjust pH. According to the pKa values of both the drugs and selected. An attempt was made in phosphate buffer instead of water in different ratio, this also gave sharp peaks. Hence potassium dihydrogen orthophosphate pH 6 : Methanol : Acetonitrile in the ratio 70:15:15 v/v/v this gave sharper peaks and it was selected as mobile phase.

The detection wavelength was measured by scanning the solution of Pregabalin and Etoricoxib in mobile phase. In UV-spectrophotometry, spectra was overlined and wavelength of maximum absorption was selected as 210 nm.

The limit of detection and the limit of quantification were determined by using slope and standard deviation and it was calculated. The system suitability parameters such as Theoretical plate, Tailing factor, Asymmetric factor and Resolution were calculated and shown in Table-1, the parameters were found to be satisfactory as per ICH guidelines.

With the optimized chromatographic conditions, stock solutions of Pregabalin and Etoricoxib were prepared in HPLC methanol and the final dilution with methanol and prepared the mixture in the concentration range 120-195 µg /ml of Pregabalin and 96 - 156 µg/ml of Etoricoxib, 10 µl of each solution was injected and records the chromatogram at 210 nm.

The chromatogram optimized given in Figure-1 the calibration curve was plotted using concentration against peak area . The procedure was repeated for three times. The correlation coefficient was found to be above 0.9997 and 0.9999 Pregabalin and Etoricoxib. The calibration graph of Pregabalin and Etoricoxib . The optical characteristics of Pregabalin and Etoricoxib shown in Table-2.

Optimized Chromatogram

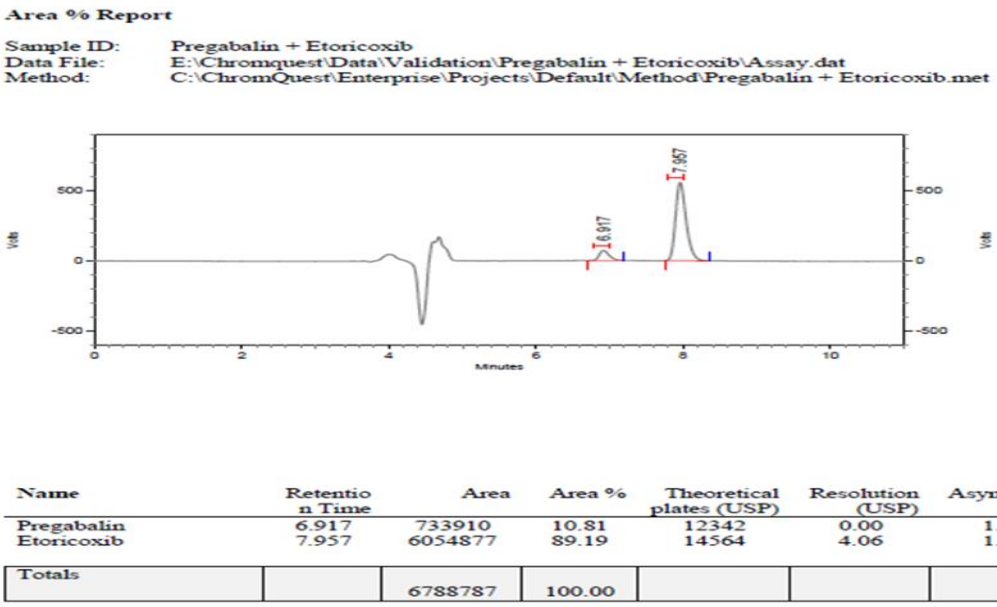

Table1: System Suitability Parameters for The Optimized Chromatogram

PARAMETERS	PREGABALIN	ETORICOXIB
Tailing factor	1.56	1.43
Asymmetrical factor	1.34	1.30
Theoretical plates	12342	14564
Theoretical plate per unit Length	227.0928	267.9776
Resolution	Between CEF and AMB 4.06	

Table 2: Optical Characteristics of Pregabalin and Etoricoxib

PARAMETERS	PREGABALIN	ETORICOXIB
Detection wavelength (nm)	210nm	210nm
Beers law limit (µg/ml)	120 - 195 (µg/ml)	96 - 156 (µg/ml)
Correlation coefficient (r)	0.9996	0.9998
(y=mx+c)	Y= (4940.5) x +(4176.5)	Y= (51409) x + (1892.7)
Slope (m)	4940.5	51409
Intercept (c)	4176.5	-1892.7
LOD (µg/ml)	0.03200470	0.00142624
LOQ (µg/ml)	0.09698395	0.00432203

Mean of six Observation

The Tablet dosage form ETOSHINE -NP TAB was selected for the analysis. The concentration 150 µg/ml of Pregabalin in the mobile phase were prepared which contains 120 µg/ml of Etoricoxib. 10 µl of each solution was injected and chromatograms were recorded. The percentage purity was found to be 99.56% of Pregabain and 100.16% of Etoricoxib respectively.

The precision of the method was confirmed by repeatability of formulation. The % RSD was found to be 0.391835, of Pregabalin and 0.639528 Etoricoxib respectively. The data is shown in Table-3.

Table 3: Results of Analysis of Tablets

Drug	Label Claim (mg)	%LABEL CLAIM Estimated*(Mean ± SD)	% RSD
PRE	75	99.56±0.3901	0.3918
ETO	60	100.16±0.6405	0.6395

*Mean of six determinations, R.S.D is relative standard deviation

Table 4: Recovery Studies of Formulation (Etoshine Np Tab)

Drug	%	Amount present* (µg mL^{-1})	Amount added* (µg mL^{-1})	Amount estimated* (µg mL^{-1})	Amount recovered* (µg mL^{-1})	% Recovery*	S.D.	% R.S.D.	S.E.
PRE	110	150	15	164.926	14.926	99.29	0.1527	0.1580	0.0088
	120	150	30	179.954	29.954	99.85			
	130	150	45	194.892	44.892	99.79			
					Mean	99.65			
ETO	110	120	12	131.9718	11.9718	99.76	0.4161	0.4160	0.0240
	120	120	24	144.131	24.131	100.5			
	130	120	36	155.921	35.921	99.8			
					Mean	99.72			

*Mean of Three observations

The accuracy of the method was performed by recovery studies to the pre analysed formulation, a known quantity of Pregabalin and Etoricoxib working standard solutions were added at different levels, injected the solutions. The percentage recovery was found to in the range between 99.50-99.81% for Pregabalin and 99.76-100.3% for Etoricoxib. The percentage RSD was found to be 0.158062 and 0.416089 for Pregabalin and Etoricoxib respectively. The low percentage of RSD values for recovery indicated that the method was found to be accurate. The values given in the Table-4, the high percentage recovery revealed that no interference was produced due to the excipients used in formulation. Therefore, developed method was found to be accurate.

All the above parameters with the ease of operations ensure that the projected methods could be applied for the routine analysis of Pregabalin and Etoricoxib pure form and in tablet dosage form.

CONCULSION:

The validated RP-HPLC method employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine RP-HPLC method of PRE and ETO in tablet dosage form instead of processing and analyzing each drug separately.

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