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HPLC-ELSD DETERMINATION OF SODIUM LAURYL SULPHATE AND POLYSORBATE IN NEBIVOLOL DRUG PRODUCT AND DIFFERENT FORMULATION PRODUCTS

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Keywords:

Nebivolol, *in-vivo*, *in-vitro*, reverse phase liquid chromatography, method development, Method validation, ICH.

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ABSTRACT: The contents of Sodium lauryl sulphate (SLS) and Polysorbate (Tween 80) were studied in different formulations of Nebivolol film coated tablets. The contents were extracted using the diluent Methanol: Water in the ratio 60:40 v/v and analyzed in HPLC coupled with Evaporative light scattering detector (ELSD). SLS and Tween 80 are solubilizing agents and the amount of these contents are critical in formulations as they are play a major role in the release pattern of the drug for in-vivo and in-vitro dissolution profiles. A reverse phase liquid chromatographic (RP-LC) method was developed for the quantification of Sodium lauryl sulphate (SLS) and Polysorbate (Tween 80). The method was optimized using buffer (prepared by dissolving 0.77g of Ammonium acetate taken in 2000mL milli-Q-water and then pH was adjusted to 4.5with dilute acetic acid solution) as mobile phase-A and Acetonitrile mobile phase-B. The flow rate was set at 1.0 mL min-1 and the column temperature was maintained at 40°C. The capability of specific method developed was demonstrated by studying the validation parameters like interferences of other sample matrix, limit of quantification, limit of detection, linearity, method precision, accuracy and robustness as per ICH. The developed method can be used for the determination of SLS and Polysorbate 80 in the regular quality control analysis for the drug product

INTRODUCTION: Nebivolol hydrochloride is available in the market with the name Bystolic as tablets for oral administration contains nebivolol hydrochloride equivalent to 2.5, 5, 10, and 20 mg of nebivolol base. In addition, BYSTOLIC contains the following inactive ingredients: colloidal silicon dioxide, croscarmellose sodium, D&C Red #27 Lake, FD&C Blue #2 Lake, FD&C Yellow #6 Lake, hypromellose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, pregelatinized starch, polysorbate 80, and sodium lauryl sulfate.

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SLS and Tween 80 are solubilizing agents and the amount of these contents are critical in formulations as they are play a major role in the release pattern of the drug for *in-vivo* and *in-vitro* dissolution profiles. There are several methods reported for the determination of surfactants, sodium lauryl sulphate using Acclaim surfactant column [1], but the separation of Polysorbate is not accounted. A method is reported for the determination of Polysorbate and sodium lauryl sulphate using charge aerosol detector and Diode array detector [2-4].

There are methods reported for the determination of Polysorbate and its related compounds by ELSD and MS detector [5] and by CAD detector in therapeutic protein formulation [6]. Titration method is available for the determination of cationic surfactants [7].

The method for the determination of Polysorbate by getting single peak is developed for better quantification process with charged aerosol detection [8]. A fast quantification method for sodium lauryl sulphate is reported in an application note with Varian ELSD detector. [9]

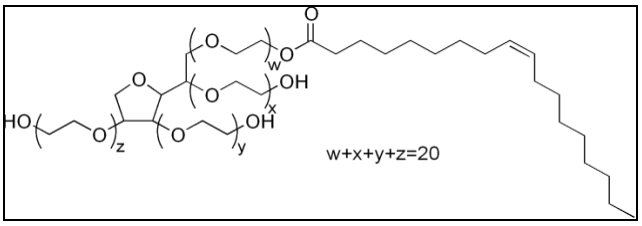

FIG.1: POLYSORBATE 80

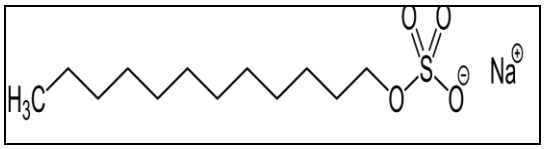

FIG.2: SODIUM LAURYL SULPHATE

The successes with ELSD detector [10] and the working principles of ELSD detector [11] are available in detail in the literature. The evaporative light scattering detector is not a spectroscopic detector, its response does not obey Beer's Law. Instead, the light-scattering phenomenon is described by three mathematical terms, all of which are influenced by particle size. The observed peak area (A) is related to the quantity of analyte on-column (m) through the relationship

$$A = a\ m^x$$

Where x is the slope of the response line and a is the response factor. Thus, logarithmic values for A and m will produce a linear trend (log A = a + x log m). This function limits the detector's ability to be used for high accuracy quantitative work.

MATERIALS AND METHODS:

Chemicals and Reagents: Polysorbate 80, Sodium lauryl sulphate, Nebivolol Film-coated tablets and Placebo mixture were obtained from AET Laboratories, Hyderabad, India. HPLC-grade of Methanol, Acetonitrile, Acetic acid and AR grade of Ammonium acetate and Milli Q Millipore (USA) purification system was used to prepare high pure water. HPLC Instrumentation and Conditions, the method development attempts and the method validation was performed in Waters 2489 LC systems with Evaporative light scattering detector.

Chromatographic conditions:

The chromatographic separation was optimized in the Develosil RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved with the buffer (0.77 gm of Ammonium acetate buffer in 2000mL of milli Q water (100%) and pH adjusted to 4.5 with diluted acetic acid solution) as mobile phase-A and Acetonitrile as mobile phase-B. The HPLC gradient program was set as (time/% mobile phase-B) 0.00/0, 5/0, 11/95, 25/95, 26/0, 35/0. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min-1 and 40°C. The ELSD detection was done with gain 50, drift tube temperature 75°C and gas pressure (Nitrogen) 40psi.A Load of 50µL injection volume used. A mixture of Methanol and Water 60:40 (v/v) was used as diluent.

Preparation of standard solutions:

A mixture of Methanol and Water 60:40 (v/v) was used as diluent. A stock solution 5mg/mL of sodium lauryl sulphate and 15 mg/mL of Polysorbate80 solution was prepared in the diluent. A series of Linearity solutions of Sodium lauryl sulphate are prepared with 0.0202mg/mL, 0.0505 mg/mL, 0.1009 mg/mL, 0.2018 mg/mL, 0.3028 mg/mL and 0.4037mg/mL. A series of Linearity solutions of Polysorbate 80 are prepared with 0.0908mg/mL,0.1816mg/mL,0.3632mg/mL, 0.5448 mg/mL and 0.7264mg/mL.

RESULTS AND DISCUSSION: Method development and optimization: The HPLC/ELSD method was optimized so as to obtain sensitive method that it could resolve sample matrix, Nebivolol from SLS and Polysorbate 80. Different stationary phases with different selectivity were used for the determination of SLS and Polysorbate80. However good single peak shapes for polymers and the resolution of all the sample matrices were achieved satisfactorily in Develosil RP-18 column with the dimension of 250mm x 4.6 mm and 5µm as particle size. A gradient elution was involved with the buffer (0.77gm of Ammonium acetate in 2000mL of milli Q water (100%) and pH adjusted to 4.5 with diluted acetic

acid solution) as mobile phase-A and Acetonitrile as mobile phase B. The HPLC gradient program was set as (time/% mobile phase- B) 0.00/0, 5/0, 11/95, 25/95, 26/0, 35/0. The flow rate of the mobile phase and the column temperature was set as 1.0 mL min-1 and 40°C. The ELSD detection was done with gain 50, drift tube temperature 75°C and gas pressure (Nitrogen) 40psi.A Load of 50µL injection volume used. A mixture of Methanol and Water 60:40 (v/v) was used as diluent.

The system suitability parameters are correlation between and Concentration in mg/mL versus area response obtained for each Sodium lauryl sulphate and Polysorbate 80 compound should not be less than 0.99. The obtained correlation coefficients are found 0.999 for SLS and 0.998 for Polysorbate80 .The developed method is specific for SLS and Polysorbate 80 in Nebivolol Film-coated tablets. The structures and chemical names of Nebivolol is given below:

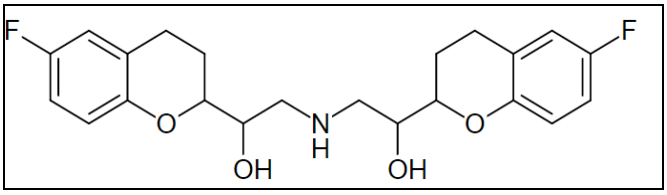

FIG.3: NEBIVOLOL 1-(6-FLUOROCHROMAN-2-YL)-{[2-(6-FLUOROCHROMAN-2-YL)-2-HYDROXY-ETHYL] AMINO} ETHANOL

Chromatograms: A typical chromatogram of Blank, Linearity solution, Placebo and Placebo-spiked sample are given below.

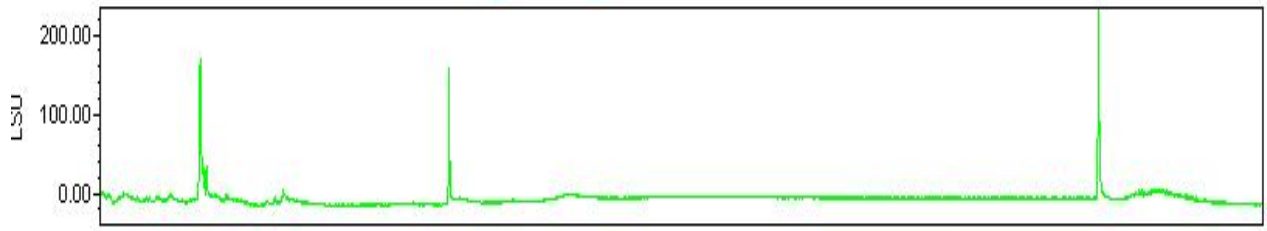

FIG.4: BLANK CHROMATOGRAM

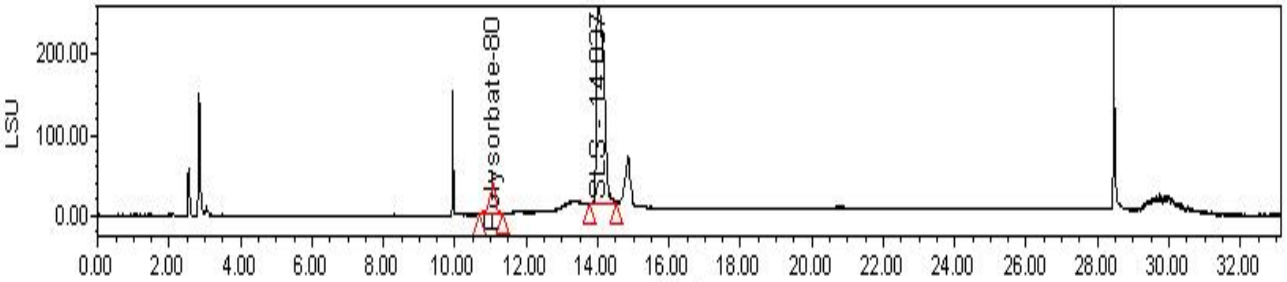

FIG.5: LINEARITY SOLUTION CHROMATOGRAM

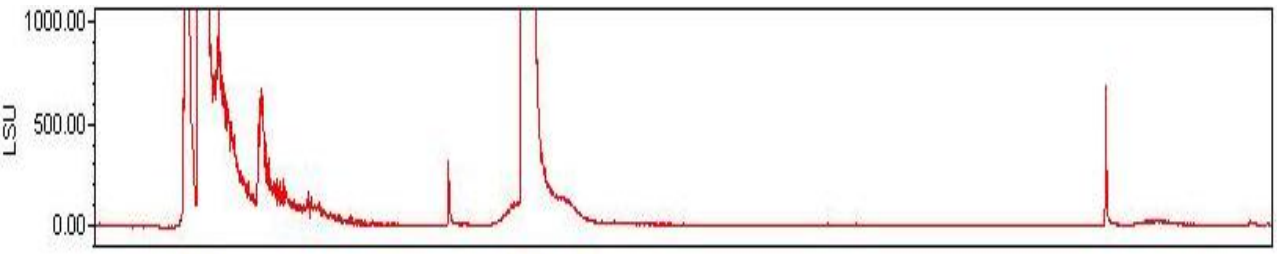

FIG.6: PLACEBO SOLUTION CHROMATOGRAM

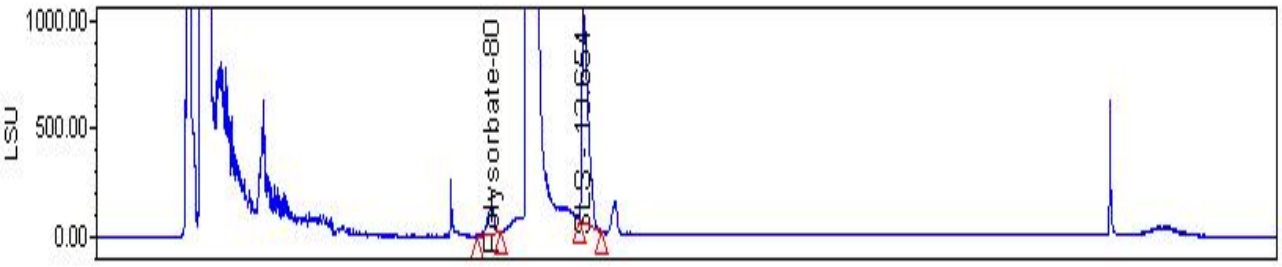

FIG.7: PLACEBO-SPIKED SOLUTION CHROMATOGRAM

Method Validation Results:

The developed method was validated as per ICH guidelines and the results are given (**Table 1**). The specificity of the developed HPLC method for SLS and Polysorbate 80 was determined in the presence of other components used in the formulation of Nebivolol Film-coated tablets. All the components of Nebivolol film-coated tablets were well resolved from SLS and Polysorbate80. The analysis was carried out by HPLC with ELSD detector signifying the specificity. The detection limit (DL) and quantification limit (QL) for SLS and Polysorbate80 compounds were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of SLS and Polysorbate80.

Linearity test solutions determination was at six concentration levels from QL to 400% of SLS and determination and Polysorbate80 was at five levels from QL to 400%. Peak area versus concentration data was performed by least-squares linear regression analysis. Also the peak area and concentrations are converted to logarithmic values and data was performed by least –squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of SLS and Polysorbate80 quantification in Nebivolol dosage form samples. The percentages of recoveries for SLS and Polysorbate 80 were calculated shown in **Table 1**.

TABLE 1: VALIDATION DATA OF THE DEVELOPED METHOD

Parameter	Sodium lauryl sulphate	Polysorbate80
DL(mg/mL)	0.0017	0.03
QL(mg/mL)	0.005	0.09
Method precision(%RSD)#	0.84	6.11
Accuracy[a](%recovery) at:		
QL	82	84
100% level	104	85
400% level	97	93

[a] Carried at QL,100% and 400% level with respect to SLS(0.10 mg/mL) and Polysorbate80(0.18 mg/mL)

The robustness of developed method was determined by altering experimental conditions slightly and evaluating the resolution between SLS and Polysorbate80. Flow rate was changed by ± 0.2units, pH was varied by ± 0.1 units, column temperature was studied at 35°C and 45°C instead of 40°C and ELSD Gas pressure was studied at 35psi and 45psi instead of 40psi in all above varied conditions the components of the mobile phase were held constant and no significant change of relative retention time was observed.

CONCLUSION: The developed specific analytical method for determination of SLS and Polysorbate80 in Nebivolol Film-coated tablets is precise, accurate, linear and specific. The validation carried out for the method in accordance with the ICH requirements are satisfactory. The developed method can be used conveniently for the routine analysis of production samples and also to check the process variability in manufacturing process of Nebivolol Film-coated tablets. The same method can also be attempted for the other drug products for the getting the information of excipients used in the process of manufacturing of drug products if specificity is proved.

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