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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF EUGENOL-AN REVIEW

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Abstract: The main objective of this study is to develop and validate technique by high performance liquid chromatography or RP-HPLC method. Determination can be reached on a RP-18 or XTerra RP18 column (250mm X 4.6mm, 5 µm), Zorbax C_{18} column , (150 mm * 4.6mm, 5µm), cosmosil C18 analytical column (150 mm X 4.6 mm, 5 m) column using a different solvent system comprising of an aggregate like methanol: water, acetonitrile: water, methanol: acetonitrile: water in isocratic elution mode{using different detector like UV, PDA and DAD} at ambient temperature along with flow rate of 1 mL/min. The developed and validated HPLC method can successfully applied for identification and quantification of eugenol. The approach may be used for assemble the characterization criteria of linearity, precision, LOD & LOQ, specificity, accuracy as per guidelines by International Conference of Harmonization (ICH).

Keywords- Eugenol, RP-HPLC, HPLC-PDA or DAD, Method validation, ICH.

I. INTRODUCTION

Eugenol is one of the important constituents in various spices such as clove, cinnamon, nutmeg, tulsi oils which are widely used as flavouring agents in foods and beverages.

Eugenol is a phenolic phytochemical present in different spices, chemically, 4-Allyl-2-methoxy phenol($C_{10}H_{12}O_2$) with molecular weight is 164.20gmol-1. Eugenol is liquid in state(at 25°C/1ATM) and colour ranges from colourless to light yellow with having the clove odor. The melting point of 9.1-9.2°C, is insoluble in water and is extremely soluble in organic solvent.[1]

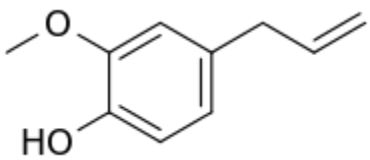

Figure no.1: Structure of Eugenol.

The in-vitro and in-vivo activities of different eugenol formulations shows the inhibited the growth of the various pathogens in agriculture field. Eugenol has a unique properties which used as a flavor or aroma ingredient in teas, meats, cakes, perfumes, flavorings, and essential oils.[2,3] It is also used as a local antiseptic and anaesthetic.[5,6] Eugenol can be combined with zinc oxide to form zinc oxide eugenol which has restorative and prosthodontic applications in dentistry. For persons with a dry socket as a complication of tooth extraction, packing the dry socket with a eugenol-zinc oxide paste on iodoform gauze is effective for reducing acute pain.[6] Eugenol-zinc oxide paste is also used for root canal sealing.[7]

Material and methods: -

Eugenol (99 %, C10H12O2) was obtained from Aldrich, USA. HPLC grade methanol was procured from Merck Specialist Private Limited (Mumbai, India). Distilled water was prepared in-house using Millipore (Millipore SA Molsheim, France). All other chemicals used were of analytical grade.

Instrumentation:

The HPLC system used for quantification of eugenol consisted of a Jasco PU-980 pump, AS-2057 auto sampler and Jasco UV-970detector. The chromatogram peaks were quantified by means of PC based Borwin software (Version 1.5). Chromatographic separation for analyte was achieved on cosmosil C18 analytical column (150 mm X 4.6 mm, 5 m) maintained at ambient temperature.

Chromatographic conditions:

Chromatographic separation can achieve using an HPLC (Water, USA) system. Mobile phase should consist of a mixture of the following solvent: water, methanol, acetonitrile, etc. the separation can be achieved on RP C18 analytical column (150 mm X 4.6 mm, 5 m). The mobile phase was pumped at a flow rate of 1mL/min. The mobile phase was filtered through a 0.45 m nylon membrane filter and degassed in an ultrasonic bath prior to use. The injection volume was 30 mL, the flow rate was 1.0 mL/min and a chromatographic peak was detected at 215nm.

Formation of Typical Stock solution:

A stock solution of 1000 ppm was prepared by accurately weighing 10 mg of eugenol standard in a 10 mL volumetric flask and it was further diluted with HPLC grade methanol up to the mark. The solution was vortexed for 10s.

Preparation of sample solution:

One g of ayurvedic formulations were taken in 10 mL of methanol and then solvent extraction was performed using a rotary shaker for 24 h. The tubes were centrifuged at 4000 rpm for 10 min and the solution was filtered with Whatman filter paper no. 41. The filtrate was collected in polypropylene tubes and stored at 4°C until further analysis. Furthermore, the filtrate was given appropriate dilution in mobile phase prior to injection on to the HPLC system.

Method Validation

The entire experimental analysis was according to the ICH guidelines and was validated for calibration curve, limit of detection, limit of quantification, system suitability, precision, accuracy, solution stability and ruggedness.

Linearity

Linearity was determined by means of calibration graph. The graph was further analyzed by using an increasing amount of each analyte and further evaluated by visual inspection of a calibration graph. These calibration curves were plotted over different concentration ranges. The absorbance of the analyte was determined at 215 nm. Regression equation was calculated by constructing calibration curves by plotting absorbance v/s concentration.

System suitability

The system performance parameters of the developed HPLC method were evaluated by six replicate analysis of the formulation at a concentration of 10ppm. The retention time of their areas were recorded subsequently. Mean area and SD was calculated to determine relative SD and the criteria for %RSD is >2% respectively.

Precision and Accuracy

Accuracy was determined for the assay method at two levels: i.e. repeatability and intermediate precision. The repeatability was evaluated by means of intraday variation and intermediate precision was determined by measuring inter-day variation in the assay method of formulation in six replicate runs. Accuracy and precision of the method assay was performed by injecting three samples spiked at 500ng/mL, 1000 ng/mL and 5000 ng/mL of drug in the placebo triplicate sets at three different levels LQC, MQC and

HQC respectively for inter day and intraday batch respectively. Mean was determined by, S.D, CV % and % nominal of three different levels was calculated.

LOD and LOQ

The limit of detection is defined as the smallest concentration that can be detected but not necessarily quantified as an exact value whereas the limit of quantification is termed as the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy that provided a peak area with signal to noise ratio higher than 10, with precision (% CV) and accuracy with (±) 10%.

Robustness

The ruggedness of the method is defined as its capacity to remain unaffected by minuscule changes in method conditions. The ruggedness was evaluated by deliberate changes in composition of mobile phase, flow rate and wavelength.

Results and Discussion

This study represents an easy and validated RP-HPLC method for estimation of eugenol. The eugenol was detected and quantified with the sample preparation stage utilizing the analytical approach proposed here employing RP-HPLC. The method validation proves its linearity, precision, LOD &LOQ and accuracy. The approach devised can be regarded a useful tool for ensuring quality control of a viable alternative to chromatography.

Conclusion

The easy, precise and delicate validated RP-HPLC method for instantaneous determination eugenol has been established. The method may be suggested for routine and quality control analysis of the investigated drugs in pharmaceutical formulations.

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