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Comparison of Analytical Techniques for Thermal Stability Analysis of Beta-Cyclodextrin for an Ebola Virus Infection Treatment

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Comparison of Analytical Techniques for Thermal Stability Analysis of Beta-Cyclodextrin for an Ebola Virus Infection Treatment

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ABSTRACT

Each New Drug Application filed with the Food and Drug Administration (FDA) must include the analytical procedures toÂ ensure the identity, strength, quality, purity, and potency of a drug substance and drug product. The BSN389 drug product (being developedÂ to treat Ebola virus infections) includes beta cyclodextrin. Evidence must be provided that the analytical procedures used in testing BSN389Â meet proper standards of accuracy, sensitivity, specificity, and reproducibility and are suitable for their intended purpose. The Bootstrap Error-adjusted Single-Sample Technique (BEST) software was used to compare the quantitative and qualitative power of IR and 1H NMR to differentiate new and partially decomposed samples of beta cyclodextrin, and the best assay will be incorporated into the thermal stability protocol for BCD.

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INTRODUCTION

Beta cyclodextrin, known simply as Î²-cyclodextrin or Î²CD or BCD, is a non-reducing cyclic oligosaccharide consisting of seven Î±-1,4-linked D-(+)-glucopyranosyl units (Figure 1). The seven membered ring is produced by enzymatic conversion of starch. This drug has applications not only in pharmaceuticals but also in the food and environmental industry. Toxins can be removed when the ring ensnares specific molecules that are targeted for removal. BCD is also a food additive that acts as a stabilizer for flavors, colors, and some vitamins.2 BCDâ€™s estimated intake is about 1-1.4 g/day and it is approved by the FDA. Researchers are now taking known information about the cyclodextrin molecule and using it as a carrier for chemotherapeutic cytotoxic anticancer drugs.3

The analytical techniques 1H NMR and infrared (IR) spectroscopy are used to measure the difference between decomposed and stable versions of BCD in this research. The Bootstrap Error-adjusted Single-sample Technique (BEST) software (see Appendix) will then identify the best analytical method to use for the thermal stability regulatory procedure for the drug.

METHODS

Preparation of Samples

Approximately two grams of beta cyclodextrin was slightly decomposed thermally by putting the sample on a Pyrex dish and placing it in a conventional oven, heating it slowly to about 232 C at a linear rate of about 28 C/5 min until the white powder sample was a slightly yellow color.

Measurements

Six separate samples of the pure and decomposed BCDs were prepared with deuterated water. H NMR spectra were recorded on a 500 MHz JOEL spectrometer, and processed with 16 scans ranging from -2 to 16 ppm. These samples were then also analyzed using a Thermo Scientific Nicolet iS10 infrared spectrometer over a wavenumber range of 4000-500 cm-1.

Analysis

The 1H NMR data were entered in TopSpin and converted into CVS files. These data along with the IR values were read into MATLAB. Each sample set of data was linked together in a variable with dimensions equal to the number of wavenumbers or chemical shifts. Each set was plotted as described in Appendix 1. The BEST program was used to determine the distance in multidimensional standard deviations (SDs) between the set of samples of pure and decomposed BCD. These values were then compared to the control distances, which were found by finding the distances between the center of the pure BCD validation spectra each pure BCD validation spectrum.

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RESULTS AND DISCUSSION

Characterization of BCD

As shown in Figure 2 and 3, the structure of BCD was characterized by 1H-NMR and IR spectroscopy. Figure 2 shows the 1H-NMR spectrum of decomposed and pure BCD. Results showed only slight left shift of the decomposed sample. Figure 3 shows a comparison of the IR spectra between the pure and decomposed drug. The decomposed sample showed a slight blue shift in the O-H stretch vibrations.

Analysis of Techniques

Table 1 shows the standard deviations output by the BEST software. The average values for the 1H NMR were higher than the average values for the IR, 6.3100 and 4.9900 respectively. This showed the differences between the stable and decomposed sample that are not obvious when shown graphically (Figures 2 and 3). The controls were expected to be less than three. There were two outliers in the NMR spectra. The outliers will be checked by LC-MS in a follow-up study.

CONCLUSION

Beta cyclodextrin samples were tested by IR and NMR. Tests showed small differences between the pure and slightly decomposed samples. The BEST software suggests that proton NMR is superior for thermal stability of BCD, but the variability is large and the outliers need to be investigated.Â Â It is worth noting that the Mahalanobis distance between the IR and NMR samples could not be calculated because the number of rows of the data matrix must exceed the number of columns. The BEST software was able to overcome this obstacle and provide a useful result.

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