Abstract

TE 132917

Drugs containing a chiral center are present in two enantiomers. Eighty percent of these drugs distributed in the market are in the combination of two enantiomers (racemic form). Pharmacodynamic, toxicity, distribution and metabolism rate of enantiomers are different. This resulted in the difference in therapeutic efficacy. An analytical method for determination of the two enantiomers is the only mean to study these effects.

In this study, Pirkle column development was reviewed. Pirkle's columns, *(R)*-N-3,5-dinitrobenzoyl-phenylglycine (DNBPG) were made. The packing material was derivatised from silica to aminopropyl silica and DNBPG was introduced at the amino part (CSP-S). This column was compared with the column packed with DNBPG synthesised from commercially available aminopropyl silica (CSP-P) and also the commerial DNBPG (ChraSep®DNBPG) chiral column. Particle sizes of all kinds of silica were determined. Packing methods using methanol and hexane as the slurry solvent were compared at various presures. Methanol was found to be a better solvent, giving a more efficient column. The efficiency in separation of racemic bendroflumethiazide by CSP-S, CSP-P and ChiraSeP®DNBPG was compared. There was difference in the efficiency which is due to the difference in structures of the chiral stationary phases. The mechanism of the chiral stationary phase and analyte, the displacement of the mobile phase on the column were propesed.

Mixture of hexane and dichloromethane (55:35) was used as a normal mobile phase and 5% of TFA was used as a solvent modifier with the ratios of 55:35:10 (MPI), 55:35:5.5 (MP2) and 55:35:3.5 (MP3). Enantiomeric separation of bendroflumethiazide was obtained with the system of CSP-S and MP1 and the system of CSP-P and MP2 and MP3

The parameters obtained from the corresponding chromatogrammes are as follows:

CSP-S with MP1 gave retention times of 14.09 and 16.07 minutes, the k' were 7.94 and 9.24, α and R were 1.14 and 1.27, respectively. CSP-P and ChiraSeP® DNBPG with MP1 gave retention times of 3.98 and 6.89 minutes and k' were 1.54 and 1.19 respectively. Number of theoretical plates were 1813 and 8117 and the height equivalent of theoretical plate were 30 and 69 μm, respectively. No enantiomeric separation was observed.

CSP-P with MP2 gave retention times of 7.64 and 8.05 minutes, k' were 3.87 and 4.11, α and R were 1.05 and 0.44 respectively.

CSP-P with MP3 gave retention times of 17.53 and 18.75 minutes, k′ were 10.16 and 10.94, α and R were 1.07 and 0.81 respectively.

ChiraSeP®DNBPG with MP2 and MP3 did not resolved the racemic.

In this study the interaction of bendroflumethiazide with the chiral stationary phase was proposed. It was also found that the chiral material was very selective. The parts that played the crucial role in separation were not only the chiral part but also the spacer (number of carbon between silica and the chiral part), and the functional group on other bond of silicon and adjacent silanol group on silica. Modification of solvent was necessary for chiral recognition.

There were differences among 3 columns. For the CSP-S the 2 groups of silanol of silica bound covalentely (ether like linkage) with the two bonds of silicon. The other two bonds of silicon is ethoxy and aminopropyl(*R*)-phenylglycine- 3,5-dinitrobenzoic acid amide linkage. For CSP-P the silicon in the commercial aminopropyl silica was bonded with 1 silanol group. The other two bonds connected to methyl groups and the fourth was bonded with aminopropyl(*R*)-phenyl glycine-3,5-dinitrobenzoic acid amide linkage. For ChiraSeP®DNBPG, complete structure of the compound was still unclear; partial structure which show chiral part was the same as those synthesised. New composition of mobile phase should be tried.

The problem of the study of the enantiomeric separation of drugs is quite difficult in term of the seeking of authentic standard enantiomer.

From this work our team gained experience in synthesis and pack the column with the available packing machine. In wet packing the column, methanol is a better solvent for packing than hexane. It is also learned the selectiveness of the column which does not depend only on the chiral part. Functional groups in the surrounding also played a prominent role.

After this work, a chiral material by imprinting a pure enantiomer on a natural polymer will be used as a stationary phase to separate its own racemic and some other racemic. Simple enantiomers will be used as a mould to avoid the problem of seeking for pure enentiomeric standard.