



Introduction

An efficient and speed up development of variable compounds has been needed in the pharmaceutical area. To prepare standard molecules or impurity compounds, preparative column should have stable under any conditions, more effective productivity and reproducibility than conventional type. Recently, new technology has been applied to semi-prep column. Its column structure is quite different from conventional type, and the column shows longer life time than conventional prep column. These advantages are offered by mainly packing material density uniformly so every performance could be improved. Now YMC introduces brand new product named 'YMC-Actus' (Axial Compression Technology with Ultimate Separation) which combines improved uniformly packing technology and YMC's various packing material. In this poster, we will show advantages of YMC-Actus columns comparing our conventional products and competitors.

Features and benefits of Axial Compression Technology

Uniformly high density packing is necessary for high efficiency and high durability of HPLC column. DAC (Dynamic Axial Compression) column is widely used for preparative separation in pilot or production scale. The piston moves inside the column and maintains an adequate compression during packing process and column operation. It allows uniformly high density packing and prevents formation of voids (Fig. 1).

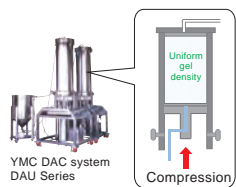


Fig.1 Mechanism of DAC system

YMC-Actus series have been developed by applying this Axial Compression Technology to semi-prep column (20 mm i. d. and 30 mm i. d.). The column bed

is compressed adequately by attaching the end assembly newly designed for YMC-Actus. It provides proper bed density (10% higher than conventional columns) and bed uniformity. The combination of technology acquired by long our experience with DAC column, the advanced technique of slurry packing, and new hardware design offers an outstanding durability and efficiency for YMC-Actus (Fig. 2).

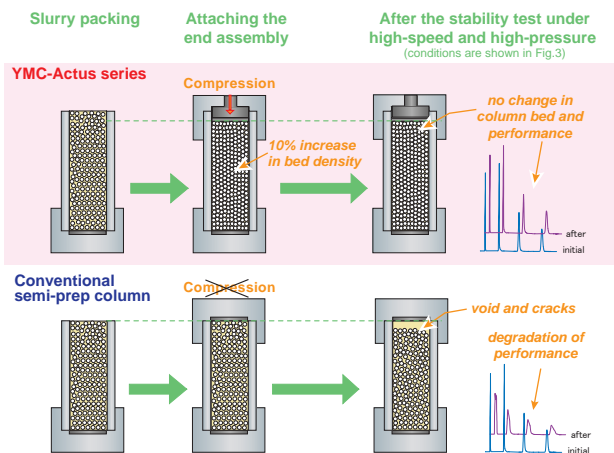


Fig.2 Packing process and bed density of YMC-Actus and conventional semi-prep column

Column durability

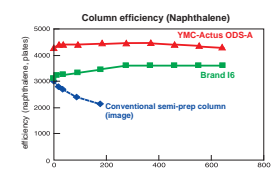
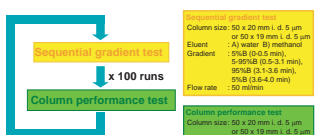


Fig.3 Stability test under high-speed gradient at variable and high pressure

In case of wrong packing conditions, rapid pressure change under the high-speed gradient condition degrades column performance. To examine bed stability, the column performance was evaluated after every 100 runs of the sequential high-speed gradient. YMC-Actus offers superior initial column efficiency compared to competitors. Furthermore, its excellent performance is maintained after a long series of gradient test (Fig.3).

As shown in overlay chromatograms of Fig. 4, YMC-Actus provides outstanding stability and reproducibility in the separation of pharmaceuticals dissolved in 100% DMSO, even after 1000 injections under the fast gradient condition.

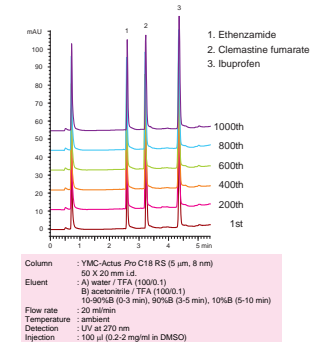


Fig.4 Stability under the condition of fast gradient and 100% DMSO injections

Scalability

YMC-Actus series is developed for laboratory scale preparative HPLC in R&D etc. Fig.5 shows column scalability of YMC products. YMC offers various column sizes from analytical scale to production scale. The analytical conditions can be directly scaled up to preparative conditions because the preparative column packed with the same packing materials provides equivalent efficiency to the analytical column.

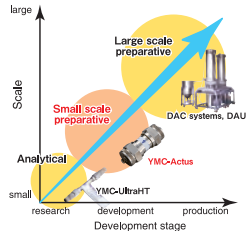


Fig.5 Scalability of YMC column

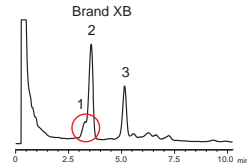
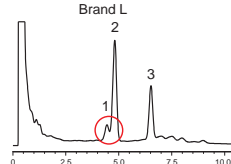
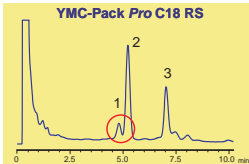
YMC-Actus series have 6 types of reversed-phase packing materials. Each of them has unique separation characteristics. The optimal selectivity is available for any purification.

Purification of hydrophobic compounds with similar structure

Capsaicinoids in red pepper

Analysis 50 x 4.6 mm i. d. 5 µm

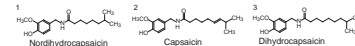
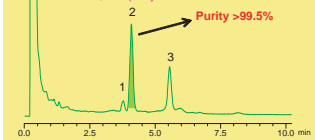
2.0 ml/min, 20 µl injection



Purification

YMC-Actus Pro C18 RS, 50 x 20 mm i. d. 5 µm

40 ml/min, 400 µl injection



Eluent	A) methanol/water/TFA (50/50/0.1) B) methanol/TFA (1000:1) 0-30%B (0-5 min), 30%B (5-10 min)
Temperature	25°C for 50 x 4.6 mm i. d. ambient for 50 x 20 mm i. d.
Detection	UV at 280 nm
Sample	methanol extract of a commercial red pepper (1 g/3 ml)

Fig.6 Analysis and purification of capsaicin in red pepper

Pro C18 RS was compared with competitors about separation of capsaicinoids in red pepper. It achieved better resolution between nordihydrocapsaicin (peak 1) and capsaicin (peak 2) than competitors (Fig.6 upper). So purification of capsaicin with YMC-Actus Pro C18 RS was examined. As the result, analytical separation could be directly scaled up to preparative separation maintaining excellent resolution, and the purity of target compound was more than 99.5% (Fig.6 under).

Pro C18 RS has superior selectivity for hydrophobic compounds that differ slightly in structure and hydrophobicity. It also has excellent stability under acidic or alkaline conditions and can be used in wide pH range. YMC-Actus Pro C18 RS is effective to fast method development for purification of various compounds.

Purification of highly polar compounds

Crude synthetic 30mer oligonucleotide (5'-CCGCTCGAGCTAAAAAGCCTGTGTACC-3')

Analysis 1.0 ml/min, 5 µl injection

Purification 19 ml/min, 100 µl injection

Hydrosphere C18

50 x 4.6 mm i. d., 5 µm

Impurities

30mer

Recovery 56%

Brand XT

50 x 4.6 mm i. d., 5 µm

Impurities

30mer

Recovery 35%

Brand XT

50 x 19 mm i. d., 5 µm

Impurities

30mer

Recovery 35%

Brand XT

50 x 19 mm i. d., 5 µm

Impurities

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30mer

Recovery 35%

Brand XT

50 x 19 mm i. d., 5 µm

Impurities

30mer

Recovery 35%

Brand XT

Eluent	A) 10 mM DBA-acetic acid (pH6.0) / methanol (60/40) B) 10 mM DBA-acetic acid (pH6.0) / methanol (20/80) 10-30%B (0-30 min)
Temperature	ambient
Detection	UV at 269 nm
Sample	synthetic oligonucleotide (100 µM)

Hydrosphere C18 was compared with competitor Brand XT about separation of highly-polar short oligonucleotide. In analytical scale, many impurities could be separated from the target product by one-nucleotide difference on Hydrosphere C18 (Fig.7 left). Even in purification scale, YMC-Actus Hydrosphere C18 gave superior separation and recovery (Fig.7 right). Hydrosphere C18 is designed for separation of highly polar compounds and can be used under 100% aqueous mobile phase conditions. So YMC-Actus Hydrosphere C18 is useful for purification of hydrophilic compounds such as oligonucleotides, organic acids, oligosaccharides and glycosides.

Purification of crude extract under fast gradient condition at high flow rate

Glycyrrhizic acid in herb medicine

Analysis

YMC-Pack ODS-AQ 50 x 4.6 mm i. d. 5 µm

3 ml/min

10 µl injection

Glycyrrhizic acid

Impurities

99.5%

After purification

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

99.5%

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99.5%

99.5%

99.5%

99.5%

99.5%

Purification

YMC-Actus ODS-AQ 50 x 20 mm i. d. 5 µm

60 ml/min

500 µl injection

Glycyrrhizic acid

Impurities

99.5%

After purification

99.5%

99.5%

99.5%

99.5%

99.5%

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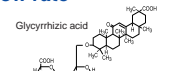
99.5%

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Conclusion

- YMC-Actus series are desirable columns for milligram scale preparative HPLC of various compounds.
- Excellent column durability and efficiency under high-throughput conditions is offered by combination of YMC's uniform packing technology acquired through long experience of DAC column and our excellent packing materials.