Waters
Lab Highlights
An Internal Communication
of Applications and Techniques
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EFFECT OF ACID/BASE WASHING ON ACCELL™ QMA MEDIA

The washing of ion exchange columns with acid and/or base is often employed to regenerate columns or as a method of cleaning columns which have seen a large number of relatively "dirty" samples.

A system was set up to examine the effects of acid and base washes on the general performance and stability of Accell™ QMA media. The chromatography of three proteins was evaluated before and after two washing procedures, a "short wash" and a "longer wash" with greater exposure of base to the ion exchanger. All operations were performed at room temperature.

Accell™ QMA media was slurry packed in a 9mm ID X 10cm glass column. A U6K injector was used for sample introduction, the gradient was generated by two M6000 Solvent Delivery Systems controlled by a 680 Gradient Controller. Detection at 280nm was monitored using a 440 fixed wavelength detector at 0.1 AUFS.

The three proteins were: cytochrome C which should not be retained under the conditions used, carbonic anhydrase which elutes early in the gradient , and ß-lactoglobulin which elutes late in the gradient. A 20µl injection contained 40µg cytochrome C, 70µg carbonic anhydrase, and 190µg ß-lactoglobulin. The flow rate was 1 ml/min with a 25-minute linear gradient from 20mM Tris (pH=8.0) to 20mM Tris (ph=8.0) with 0.375M NaCl.

The "short wash" procedure was performed at 1 ml/min and started with 4 column volumes of water then 8 column volumes of 0.1N NaOH, 6 column volumes of water, 8 column volumes of 0.1N HCl, and finally 6 column volumes of water.

The "longer wash" procedure exployed the same sequence as the short wash but was operated at 0.5 ml/min for longer exposure to acid and base rather than the 1ml/min employed in the "short wash" procedure, and the sequence was repeated twice for the "long wash" procedure. The column was then washed at 0.5 ml/min with the 0.1N NaOH for an additional 50 column volumes. The column in the 0.1N NaOH was allowed to sit for another 8 hours before evaluation.

Results

Accell™ QMA media will tolerate limited exposures to base and acid without significant changes in the chromatography (Figure 1a and 1b). However, after the "long wash" and exposure procedure, the column did develop a void, and the chromatography of cytochrome C and the tailing peaks of carbonic anhydrase appeared to be affected even after the void was taken up (Figure 2a and 2b).

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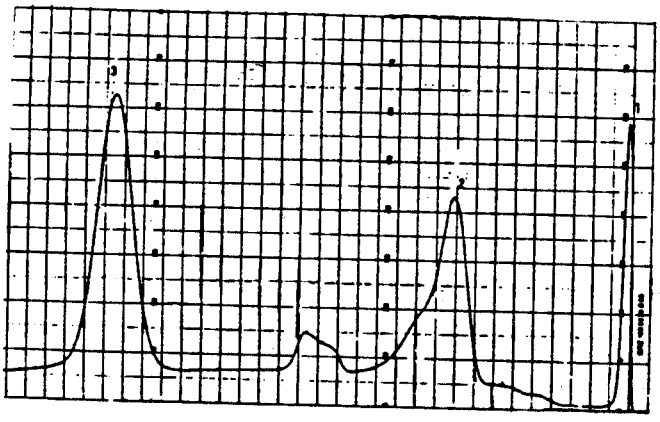


Figure 1a. Chromatogram of cytochrome C (1), carbonic anhydrase (2), and ß-lactoglobulin (3) before the short washing procedure.

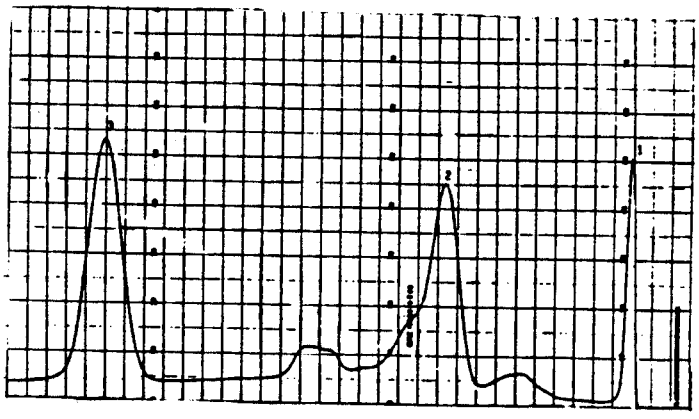


Figure 1b. Chromatogram after the short wash procedure showing little change in the chromatography.

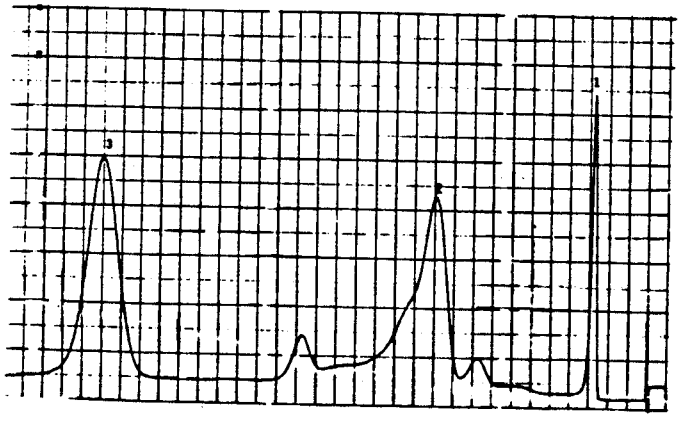
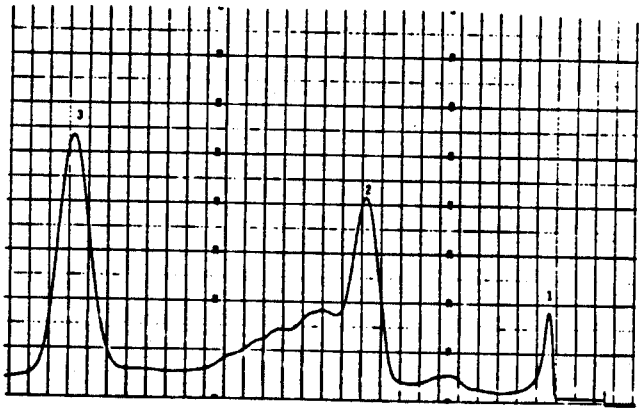


Figure 2a. Chromtogram before the long washing and exposure procedure.



Fibure 2b. Chromatoghram after the long washing and exposure procedure showing some loss of resolution.

Clearly, the degree of permissible chromatographic degradation caused by a washing procedure is dependent upon the type of separation being performed and the resolution required from that separation.