Stability-indicating analytical approach for lactoferrin

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INTRODUCTION

Lactoferrin is a globular glycoprotein with a molecular mass of about 80 kDa that is widely represented in various secretory fluids. As the main iron-binding protein in human milk it has anti-microbial, anti-inflammatory, immunomodulatory, anticancer and many other biological activities[1]. Protein characteristics demand a complex analytical approach and special handling in stability studies[2].

OBJECTIVE

The aim of our work was to develop an analytical methodology for stability evaluation of lactoferrin in preformulation studies as well as in final products.

EXPERIMENTAL

Bovine lactoferrin was obtained from Sigma-Aldrich. Commercially available dietary supplements containing lactoferrin were also used. Methods were optimized on various reversed-phase (RP) and size exclusion (SEC) stationary phases. An Agilent 1100/1200 HPLC system, equipped with a diode array detector was used.

RESULTS

Our analytical methodology was focused on two complementary chromatographic methods. Among various types of RP columns tested, BioZen Intact XB-C8, 150×4.6 mm, 3.6 µm (Phenomenex) showed the best performance. The optimised RP method utilised a shallow gradient using 0.1% TFA in water and acetonitrile (Fig. 1).

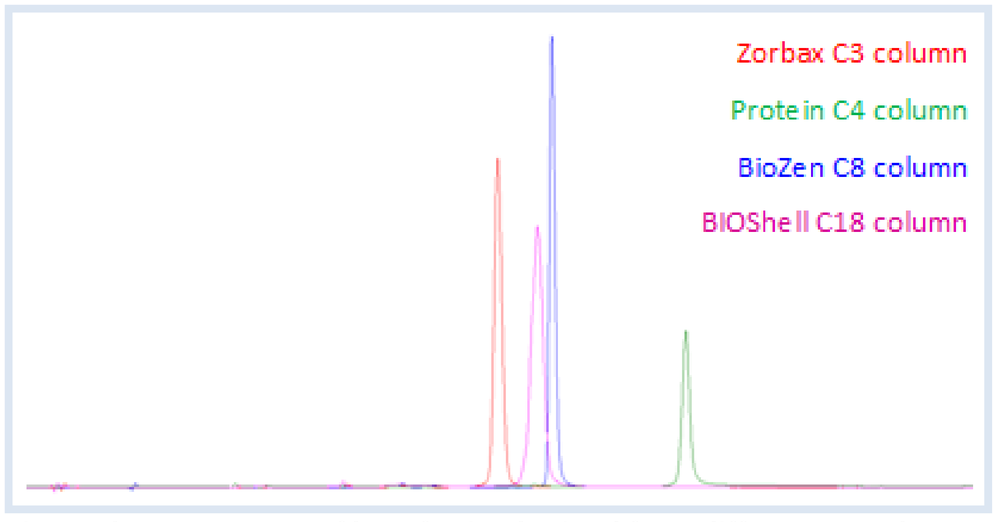

Fig. 1: Chromatograms of lactoferrin obtained from different RP columns.

The optimised SEC method was comprised of an XBridge Protein BEH SEC 150×7.8 mm, 3.5 µm column (Waters) and phosphate buffer with addition of NaCl as mobile phase (Fig. 2).

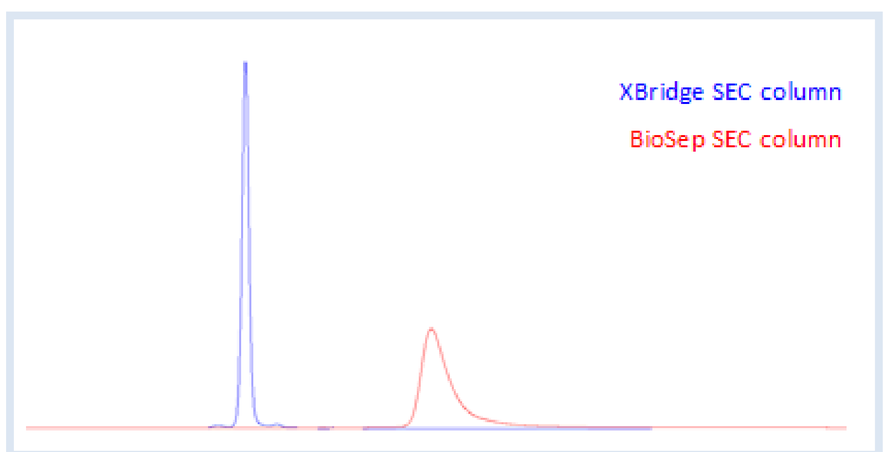

Fig. 2: Chromatograms of lactoferrin obtained from two different SEC columns.

The stability-indicating nature of the chromatographic analytical approach was proven by stress testing, where it was shown that lactoferrin is most prone to degradation under thermal and alkaline conditions. The optimised methods were successfully validated according to ICH guidelines and applied to stability studies.

Both methods were also used for lactoferrin determination in commercial products (Fig. 3) and showed a certain degree of disagreement between the obtained results. In addition to other advantages, RP method is more appropriate for lactoferrin quantification as it was noticed that the sample media may substantially affect the SEC chromatographic response.

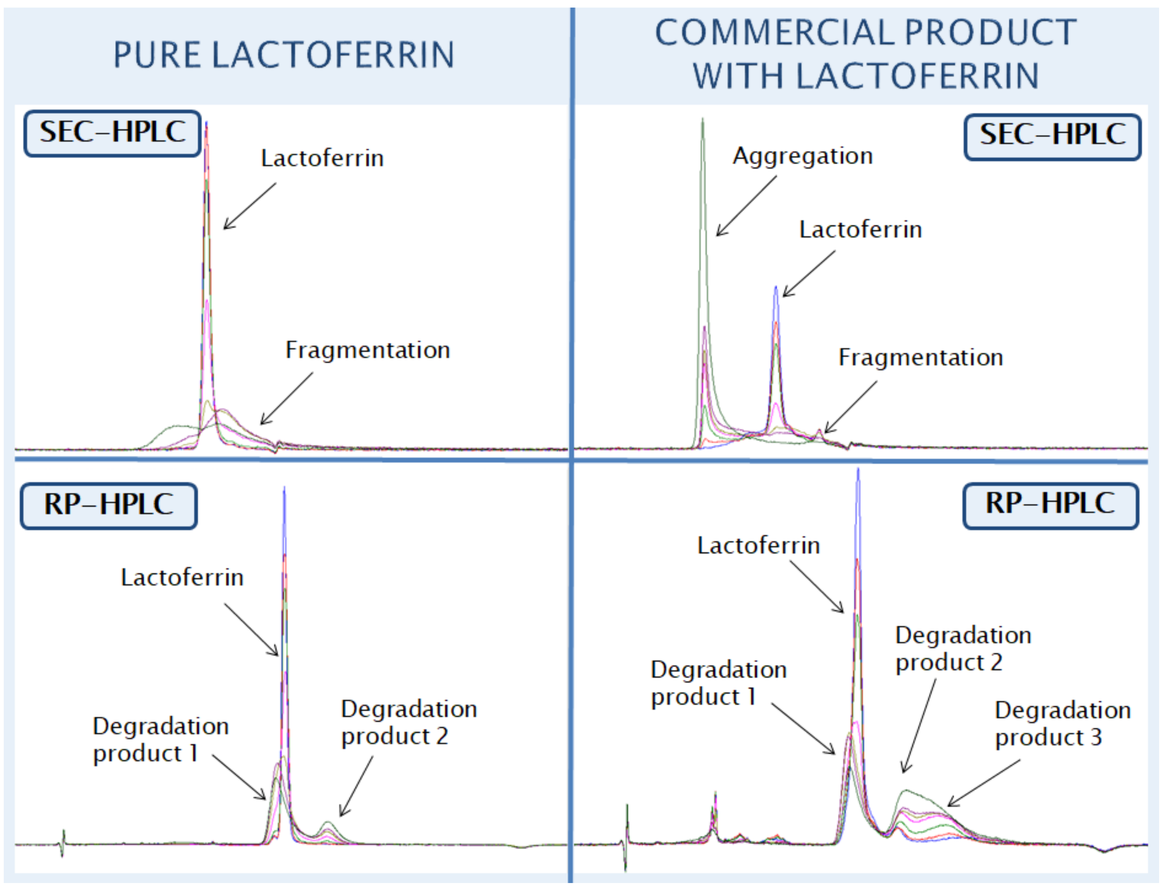

Fig. 3: Chromatograms of pure lactoferrin and commercial product with lactoferrin stored for 0 min, 15 min, 30 min, 60 min, 120 min, 240 min and 24 h at 60 °C.

CONCLUSIONS

A stability-indicating analytical approach was established. In addition to both optimized chromatographic methods, the complementary information from spectroscopic methods (fluorescence, UV-spectroscopy, total proteins) was also included to support the thorough and more comprehensive evaluation of lactoferrin stability.

ACKNOWLEDGMENT

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