



HAL
open science

A simple method to quantify lactoferrin in milk by Micro-Batch Resin Extraction (MBRE) before RP-HPLC

Sylvie Pochet, Céline Arnould, Perrine Poton, Jocelyne Guinard-Flament,
Eric Beuvier

► To cite this version:

Sylvie Pochet, Céline Arnould, Perrine Poton, Jocelyne Guinard-Flament, Eric Beuvier. A simple method to quantify lactoferrin in milk by Micro-Batch Resin Extraction (MBRE) before RP-HPLC. XIIIth International Conference on Lactoferrin: Structure, Function and Application, Nov 2017, Rome, Italy. hal-03678911

HAL Id: hal-03678911

<https://hal.science/hal-03678911>

Submitted on 25 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

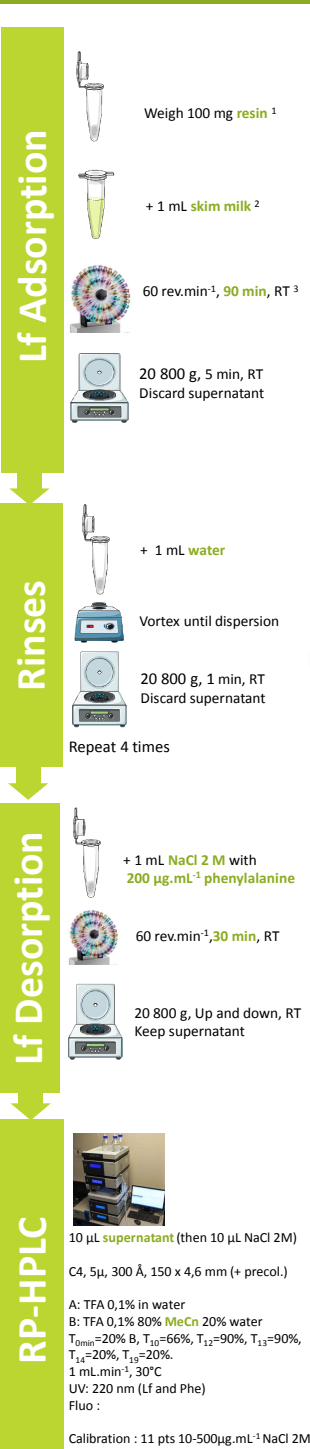
A simple method to quantify lactoferrin in milk by Micro-Batch Resin Extraction (MBRE) before RP-HPLC

Sylvie Pochet^{a*}, Céline Arnould^a, Perrine Debournoux^b, Jocelyne Guinard-Flament^b, Eric Beuvier^a

^a INRA, UR 342-Technologie et Analyses Laitières, 39801, Poligny, France

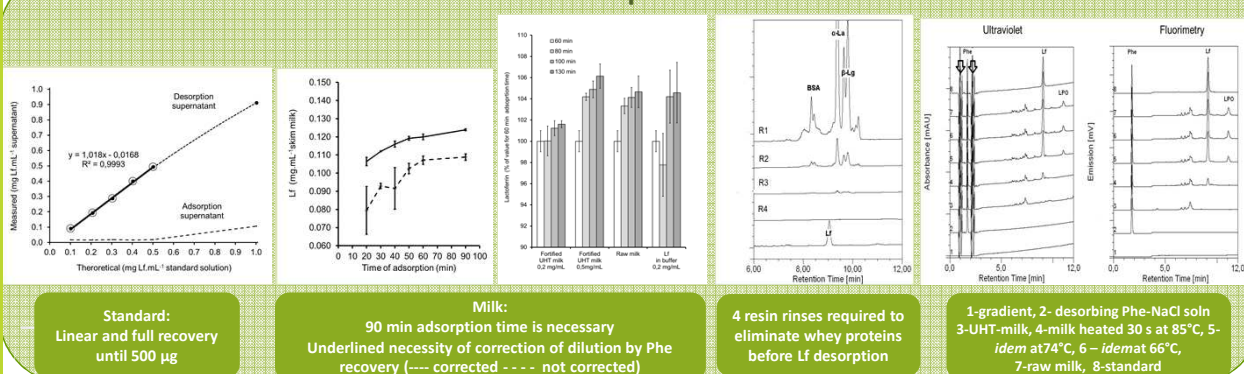
^b INRA, UMR 1348-PEGASE, Agrocampus-Ouest, 35000, Rennes, France

* Corresponding author: sylvie.pochet@inra.fr



Separative techniques (HPLC, capillary or SDS-PAGE) were rarely used for the quantification of lactoferrin (Lf) in milk compared to immunochemical methods (ELISA, RID, biosensing) which were found more specific and sensitive, but also more expensive and moderately reproducible. We present here a new HPLC-based method validated for the quantification of lactoferrin in bovine milk. Lactoferrin, due to its cationic character (pI > 8), is positively charged at the pH of native milk (6,8-7) whereas almost all caseins and whey proteins (pI < 5,6) are negatively charged. This difference is used to specifically and quantitatively adsorb Lf on a strong cation-exchange resin assay developed at a micro-batch scale. Lf is then desorbed increasing ionic strength without any dilution as compared to its concentration in milk. Finally, Lf is fully separated from other co-extracted minor cationic substances and quantified by conventional RP-HPLC with UV detection. The extraction using resin replaced the acid precipitation of caseins which was used in most HPLC-based methods causing loss of lactoferrin and keeping whey proteins in sample extract that interfered with Lf in HPLC.

Some data on the development of the method



- All combinations of concentration of NaCl (2, 2.25 and 2.5 M) in desorbing solution and time of desorption (30, 60 and 90 min) gave non statistically different results.
- Thirty samples can be extracted each day (limited by the centrifuge capacity) and HPLC analyses are then performed automatically (Lf in desorption solution was stable at least 10 h on the autosampler at the 12°C).

In-lab validation by accuracy profile ISO 5725

Fortified UHT bovine milk, 6 levels, 3 replicates/day, 5 days. See Feinberg, 2007 for calculations

		Levels						
		K1	K2	K3	K4	K5	K6	
UV	Concentration (target)	28.4	56.4	103.3	207.1	306.6	514.4	
	Accuracy Concentration (measured)	31.5	59.1	106.3	209.3	307.5	504.9	
	Recovery rate	110.9	104.9	103.0	101.1	100.3	98.1	
	Precision	Within-day RSD (S _v)	1.16	1.97	2.67	1.33	1.78	0.91
	Between-days RSD (S _b)	3.35	1.93	0.00	1.59	1.67	1.47	
	Intermediate RSD (S _s)	3.55	2.76	2.67	2.08	2.44	1.73	
	Expanded uncertainty (U)	8.57	6.15	5.68	4.49	5.20	3.65	
Fluorimetry	Accuracy Concentration (measured)	31.7	59.9	107.0	208.6	304.5	503.6	
	Recovery rate	111.6	106.2	103.6	100.7	99.3	97.9	
	Precision	Within-day RSD (S _v)	1.31	4.61	2.57	3.13	2.66	0.80
		Between-days RSD (S _b)	13.7	5.98	4.64	2.86	6.40	7.84
	Intermediate RSD (S _s)	13.7	7.55	5.31	4.24	6.93	7.88	
	Expanded uncertainty (U)	33.5	17.2	11.9	9.07	15.0	16.9	

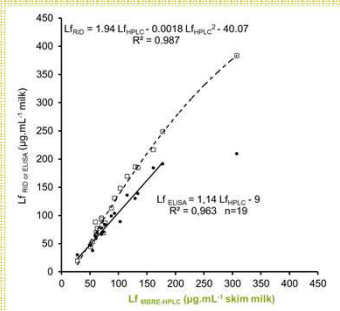
Recovery (111 to 98 %), precision (intra-day : <3%, inter-day : <4%)
 LOD_{5%} = 2 µg/mL, LOQ_{5%} = 6,7 µg/mL
 but taking account of precision (accuracy profile) : LOQ = 25 µg/mL (λ=20%, β=95%)
 Fluorimetric detection is not recommended because of poor reproducibility

Conclusions

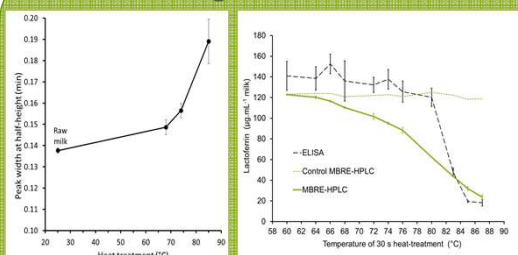
The proposed method is simple, flexible, cheap, specific, sensitive and accurate. It requires no particular skill for good precision. It is an improvement over previously published HPLC-based methods as being more accurate (no loss of lactoferrin due to sample preparation and no interference with whey proteins) and is more sensitive (no dilution during sample preparation).

The method competes well with ELISA-based method considering especially its lower cost and better precision. The method was developed using bovine milk (50-350 µg.mL⁻¹ Lf in the literature) but could probably be applied to other milk species and other fractions such as colostrum or acid serums or of cheese by means of an adaptation of the resin / sample ratio and some checks.

Comparison with immunological methods



Effect of heating milk on Lf denaturation



Lf peak is widening with milk heating. Peak surface must be used (and not height)

MBRE-HPLC gives different information about heat-denaturation of Lf in milk as compared to monoclonal ELISA

¹- SPEC 70 SLS (Pall Biosepra) : semi-rigid microbeads of acrylic copolymer (260 to 600 µm) grafted with sulfopropyl residues, binding capacity: 20 mg Lf.mL⁻¹. Equilibrated in 20 mM phosphate buffer pH 6,8. See also Liang et al., 2011. Int. J. Env. Res. and Public Health, 8 3764-3776.

²- 1000 g, 30 min, 4-5°C

³- RT: Room Temperature (22,5°C ± 1)

