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**Carolina Lyrio Tenorio-Daussat**

# Peptide-labeling with metals using MS detection and optimization of metalloprotein extraction procedures in biological samples with proteomic purposes

**TESE DE DOUTORADO**

Thesis presented to the Programa de Pós-graduação em Química of the Departamento de Química, PUC-Rio as partial fulfillment of the requirements for the degree of Doutor em Química.

Advisors: Prof. Tatiana D. Saint'Pierre
Prof. Dirk Schaumloffel
Co-Advisor: Dr. Rachel Ann Hauser-Davis

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Para Deus.
Para meus pais, Claudio e Tania, que me deram vida e força.
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# Abstract

Tenorio-Daussat, Carolina L.; Schaumlöffel, Dirk; Saint'Pierre, Tatiana D. **Peptide-labeling with metals using MS detection and optimization of metalloprotein extraction procedures in biological samples with proteomic purposes.** Rio de Janeiro, 2014, 164 p. PhD Thesis – Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro and Institut des Sciences Analytiques et de Physico-Chimie pour L'Environnement et les Matériaux (IPREM), Université de Pau et des Pays de l'Adour.

This PhD thesis is a Cotutelle between the PUC-Rio (Brazil) and the UPPA (France).

This work developed a new method for the identification and quantification of peptides, by optimizing some of the available strategies suitable for labeling peptides with lanthanide metals with subsequent separation by nano-HPLC with UV detection, matrix-assisted laser desorption ionization-mass spectrometry (MALDI MS). First, peptides were labeled with the three different lanthanide metals using a functional DOTA-based reagent. The results demonstrate that the derivatization reaction using the chelating reagent DOTA-NHS-ester was effective for single peptides and peptide mixtures, verified from the m/z relation obtained by MALDI MS. In parallel, environmental analyses were conducted, by performing the standardization of metalloprotein purification in fish bile, since this matrix has been reported as a biomarker for environmental metal contamination. Different procedures and reducing agents were applied to purify MT isolated from fish (Oreochromis niloticus) bile and liver. Spectrophotometrical analyses were used to quantify the resulting MT samples, and SDS-PAGE gels were used to qualitatively assess the different procedure results. A response surface methodology was applied for bile samples. In an environmental context, biliary MT was lower than liver MT, and, bile MT seems to be more adequate in environmental monitoring scopes.

## Keywords

Peptides; nano-HPLC-ICP-MS; MALDI MS; SDS-PAGE; MT; fish bile.

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# Resumé


Tenorio-Daussat, Carolina L.; Schaumlöffel, Dirk; Saint'Pierre, Tatiana D. **Le marquage des peptides avec des métaux et détection par MS et l'optimisation des procédures de l'extraction de métalloprotéines dans les échantillons biologiques à des fins de protéomique.** Rio de Janeiro, 2014, 164 p. Thèse de Doctorat – Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro et Institut des Sciences Analytiques et Physico-Chimie pour l'Énvironnement et les Matériaux (IPREM), Université de Pau et des Pays de l'Adour.

Cette thèse de doctorat est résultat d'une cotutelle entre la PUC-Rio (Brésil) et l'UPPA (France).

Ce travail a développé une nouvelle méthode pour l'identification et la quantification des peptides, par l'optimisation de certaines stratégies disponibles appropriées pour le marquage des peptides avec des métaux lanthanide, une séparation par nano-HPLC et détection UV, et suivi par MALDI MS. Tout d'abord, les peptides ont été marqués avec les trois métaux lanthanides différents et un réactif fonctionnel - DOTA. Les résultats montrent que la réaction de transformation en dérivé à l'aide du réactif chélateur DOTA-NHS-ester a été efficace pour des peptides individuels et des mélanges de peptides, vérifiées à partir de la relation m/z obtenue par MALDI MS. En parallèle, nous avons effectué l'optimisation pour la purification de métalloprotéine dans la bile de poisson, qui est signalée entant que biomarqueurs de contamination métallique de l'environnement. Des procédures différentes et les agents réduisant ont été apliqués pour purifier les MT isolées de la bile et du foie des poissons (Oreochromis niloticus). Des analyses spectrophotométriques ont été utilisées pour quantifier les échantillons de MT, et le gel SDS-PAGE a été utilisé pour évaluer qualitativement les différents résultats de la procédure. Chaque procédure a en suite été évaluée statistiquement, une méhtode des surfaces de réponse a été appliquée. Les MT de la bile semblent être plus adéquate pour la surveillance de l'environnement en ce qui concerne l'exposition récente à des xénobiotiques qui peuvent influer sur l'expression protéomique et metalloproteomique de cette matrice biologique.



## Mots clefs

Peptides; nano-HPLC-ICP-MS; MALDI MS; SDS-PAGE; MT; bile de poisson.



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# Resumo


Tenorio-Daussat, Carolina L.; Schaumlöffel, Dirk; Saint'Pierre, Tatiana D. **Marcação de peptídeos com metais usando detecção por MS e otimização de procedimentos de extração de metaloproteínas em amostras biológicas com propósitos proteômicos.** Rio de Janeiro, 2014, 164 p. Tese de Doutorado – Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro e Instituto de Ciências Analíticas e Físico-Química Ambiental e Materiais (IPREM), Universidade de Pau e dos Países do Adour.

Esta tese de doutorado é resultado de uma cotutela entre a PUC-Rio (Brasil) e a UPPA (França).

Método de identificação e quantificação de peptídeos, através da otimização de estratégias para a marcação de peptídeos com metais e subsequente separação por nano-HPLC-UV, MALDI MS. Primeiramente, peptídeos foram marcados com 3 diferentes metais lantanídeos usando um reagente funcional NHS-DOTA. Os resultados demonstraram que a reação de derivatização usando o reagente quelante DOTA foi eficiente para peptídeos simples e misturas dos mesmos, verificada através do MALDI MS a partir da relação m/z. Em paralelo, análises ambientais foram realizadas pela otimização de um procedimento de extração de metalotioneína em bílis de peixe, uma vez que esta matriz tem sido reportada como um biomarcador ambiental de contaminação por metal. Diferentes procedimentos e agentes de redução foram aplicadas para a extração de metalotioneína em bílis e fígado de peixe *(Oreochromis niloticus.* Análises espectrofotométricas foram realizadas a fim de quantificar os extratos de MT, e gel SDS-PAGE foi usado para avaliação qualitativa dos diferentes procedimentos usados. Cada procedimento foi avaliado estatisticamente. Metodologia de superfície de resposta foi aplicada para amostras de bílis, a fim de avaliar a resposta desta matriz. Em um contexto ambiental, concentrações de MT biliar foi mais baixa que MT do fígado, no entanto, a primeira mostrou-se mais adequada para um monitoramento ambiental.



## Palavras-chave

Peptídeos; nano-HPLC-ICP-MS; MALDI MS; SDS-PAGE; MT; bílis de peixe.



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## Acronyms List

| | |
|---|---|
| 1D SDS PAGE | Sodium dodecyl sulphate polyacrylamide gel electrophoresis in 1 dimension |
| 2D SDS PAGE | Sodium dodecyl sulphate polyacrylamide gel electrophoresis in 2 dimensions |
| ACN | Acetronitrile |
| BSA | Bovine serum albumin |
| CE | Capillary electrophoresis |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DOE | Design of experiments |
| DOTA | 1,4,7,10-tetraazacyclododе- cane-1,4,7,10-tetraacetic acid |
| DTNB | 5-(3-Carboxy-4-nitrophenyl)disulfanyl-2-nitrobenzoic acid |
| DTPA | Diethylene triamine pentaacetic acid |
| DTPAA | Diethylenetriaminepentaacetic acid anhydride |
| DTT | Dithiothreitol |
| EDTA | Ethylenediamine tetraacetic acid |
| ESI | Electrospray ionization |
| FIA | Flow injection analysis |
| FT-ICR | FourrierTransform Ion Cyclotron Resonance |
| FT-IR | Fourrier Transform Infra-red |
| GSH | Glutathione |
| HFBA | Heptafluorobutyric acid |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| MAL DOTA | Maleimidocysteineamido-DOTA |
| MALDI TOF MS | Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry |
| MMTS | S-methyl methanethiosulfonate |
| MT | Metalothionein |
| MW | Mass Weigth |

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| | |
|---|---|
| NANO HPLC | Nano High Performance/Pressure Liquid Chromatography |
| NHS DOTA | N-terminus amino groups DOTA |
| PES | Protein Express Signature |
| RNA | Ribonucleic acid |
| TCEP | Tris(2-carboxyethyl)phosphine |
| TEAA | Triethylammonium acetate |
| TFA | Trifluoroethanoic acid |
| UV-VIS | Ultraviolet–visible |
| α-CHCA | α-cyano-4-hydroxycinnamic acid |

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