



Evaluation of methods for quantification of endogenous metabolites in blood serum: application in the study of potential tumor biomarkers.

Flávia P. Fillietaz*, Adriana T. Godoy, Marcos N. Eberlin, Ana Valéria C. Simionato

Abstract

Quantification of metabolites in blood serum is a challenge due to the presence of endogenous compounds in the sample matrix. Therefore, a thorough evaluation for method validation is required. In this work, different matrices were evaluated: synthetic serum, blood serum charcoal stripped and blood serum with standards addition. Matrix effect was assessed to determine the best method for quantification of endogenous metabolites.

Key words:

Metabolomics, quantification, endogenous, LC-MS.

Introduction

Metabolomics, science that studies the metabolites in a biological system, has been applied in the investigation of possible tumor biomarkers, facilitating the diagnosis and monitoring of disease¹. For this purpose, quantification of nucleosides in blood serum may be assessed by a target metabolomics approach. However, nucleosides are endogenous metabolites, posing a challenge for a trustworthy method validation, since a blank sample matrix is not available². In this work, evaluation of the validation methods were performed in three different blood serum matrices, according to the matrix effect.

Results and Discussion

For comparison of the validation processes, three different matrices for blood serum nucleosides analysis were evaluated: synthetic serum (PBS – phosphate buffered saline + 20% BSA – bovine serum albumin), pooled blood serum and blood serum charcoal stripped. After fortifying the different matrices with the same analytes concentrations for each point of the calibration curve, samples were treated with cold methanol (3:1) for proteins precipitation. The synthetic matrix and the charcoal treated serum were used to quantify the nucleosides by the internal standard method. Samples were analyzed by liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS) (Agilent 1260 Infinity Binary, 5500 Q-Trap® Sciex) operating in a MRM mode (multiple reaction monitoring) with electrospray ionization, which is one of the best techniques of quantification due to sensitivity, selectivity and speed.

The matrix effect includes the extraction efficiency after protein precipitation and the signal in LC-MS/MS. For the evaluation of the matrix effect, the calibration curves of charcoal stripped blood serum and synthetic serum were compared with the curve of pooled serum samples with addition of standards for nine nucleosides standards, and the respective slopes were assessed. The synthetic serum showed the closest curves slope (94% to 106%) to the blood serum slopes for eight nucleosides. Image 1 shows the results for guanosine).

Working with charcoal showed some disadvantages, such as incomplete removal of some analytes from serum, the use of large amounts of biological matrix and the difficult removal of the charcoal residue in serum after treatment, which may have caused the decrement of analyte

concentration and, consequently, reduction of analyte signal.

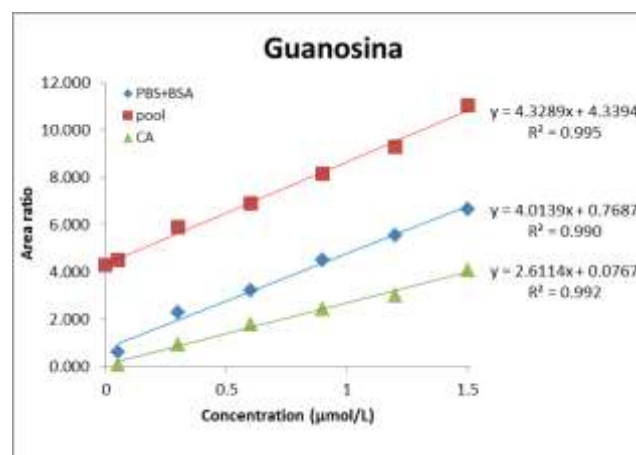


Image 1. Analytical curves of guanosine in serum by addition of standards (pool), synthetic serum (PBS+BSA) and blood serum charcoal stripped samples (CA).

Conclusions

According to the results and the evaluation of matrix effect for nine nucleosides standards, the best method for quantification of these endogenous metabolites in blood serum is the use of the synthetic serum, due to the smallest matrix effect, indicating that this matrix is the closest to the biological matrix. Besides, working with a blank matrix for quantification of metabolites results in more reliable concentrations. The blood serum charcoal stripped matrix must be further investigated to be applied in validation methods.

Acknowledgement

To FAPESP (proc. nº 2016/07014-5) and CNPq.

¹ Atkinson, A. J., Colburn, W. A., DeGruttola, V. G., DeMets, D. L., Downing, G. J., Hoth, D. F. *Clinical Pharmacology and Therapeutics* **2001**, 69(3), 89–95.

² Thakare, R.; Chhonker, Y. S.; Gautam, N.; Alamoudi, J. A.; Alnouti, Y. J. *Pharma. Biomed. Anal.* **2016** 128, 426.

³ Ghassabian, S.; Griffiths, L.; Smith, M. T. J. *Chromatog. B* **2015**, 1000, 77.

⁴ Agência Nacional de Vigilância Sanitária (ANVISA), Resolução RDC nº 27 de 17/05/2012.