DOWNLOAD PDF CHROMATOGRAPHY AND MODIFICATION OF NUCLEOSIDES, PART B

Chapter 1 : chromatography of alkaloids part b | Download eBook PDF/EPUB

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Tumor biomarkers allow earlier and less invasive diagnosis, hence they are vital in the cancer treatment. Nucleosides have been investigated as a potential group of tumor biomarkers present in biological fluids. In this work, a method for the analysis of nucleosides in blood serum samples by capillary electrophoresis with UV detection CE-UV was developed and validated according to the current legislation in Brazil. Separation of ten nucleosides plus the internal standard was achieved in ca. The method may contribute for earlier and more accurate diagnosis of cancer cases. Introduction Cancer has become a condition of great concern all over the world. One in each two human beings will develop some type of malignant tumor during lifetime, but one third of death cases could be avoided if the disease was detected and treated early, when cancer is restricted to one organ and metastasis has not initiated. However, tumors are generally asymptomatic in such phase, so a test or exam that identifies the disease in the initial stage and that is noninvasive, simple, safe and easy to be performed would be ideal. Some tumor biomarkers, synthesized within cancer development, may be detectable right in the beginning of the disease, constituting an ideal system for early diagnosis. The identification of biomarkers for cancer diagnoses has been widely researched due to the possibility of early detection and achievement of data about the pathology base and the stage of the disease. Different tumor biomarkers can be found for different types of cancer, and levels of the same tumor biomarker can be altered in more than one type of cancer. Tumor biomarkers can be used in conjunction with other tests, e. In the post-transcpitional stage of transfer ribonucleic acid tRNA , the four common nucleosides i. Unlike normal nucleosides, the modified molecules cannot be reincorporated into nucleic acid chains because of the absence of suitable enzymes, being eliminated in body fluids. As a result, increased levels of nucleosides both modified and non-modified are excreted from cancer cells. Therefore, investigation of such analyte profile is of outcome importance since it may assist the early diagnosis and cancer treatment of patients, therefore increasing the survival rates. Publications have confirmed that the levels of some nucleosides are affected by tumor development, as shown in Tables 1 and 2 , but the degree of such alteration in blood serum and the relation to cancer base pathologies have not been stated yet. The differences presented in published data emphasizes the necessity of further studies. Nevertheless, the practical application of nucleoside analyses as a diagnostic tool still requires improvements of methodologies, validation and expertise. Other techniques, such as high-performance liquid chromatography HPLC , have been used to analyze nucleosides in biological materials, but CE usually presents higher resolution power and less solvent consumption, which makes it more suitable for routine analysis. The validation steps were entirely executed in blood serum from healthy subjects, emphasizing the novelty of the method, since few works have reported the validation of this method employing the biological matrix or have presented the complete procedure used for the evaluation of the figures of merit. The combination of the developed method with the ones routinely used in clinical analysis may corroborate for more accurate diagnoses of early stage cancer and for aiding the detection of false-positive or false-negative results. Experimental Reagents and solutions All reagents used herein were of analytical purity grade. Ten nucleoside standards cytidine: Nucleoside standard solutions were prepared by weighting the necessary amount of the standards and dissolving in water with the aid of ultrasound for about 20 min. The solutions were kept frozen and protected from light, and were discarded every 30 days. For method development, 0. BGE solutions under all conditions described on the following sections were kept in refrigerator for about 3 months. Capillary electrophoresis Two CE-UV systems were employed for sample analyses and method development: In the first use, capillaries were conditioned by flushing 1. At the beginning of the day, capillaries were prepared by flushing with 1 mol L-1 NaOH for 15 min, water for 10 min

and BGE for 15 min. A simple conditioning procedure was applied between runs, and was constituted by 1 mol L-1 NaOH for 1 min, water for 1 min and BGE for 2 min. The capillaries were rinsed with water for 20 min at the end of the day and dried with flush of air for 10 min. Samples were injected under a pressure of 50 mbar for 15 s for nucleoside standards consuming The parameters evaluated during the optimization were: BGE pH and composition, surfactant and organic modifier concentration and applied voltage. Blood serum sample preparation Blood serum samples for the validation step were obtained from healthy male volunteers, because the method will be applied to the analyses of samples obtained from subjects with prostate cancer. Such biomolecules are tumor biomarkers commonly applied in diagnosis procedures. Therefore, the results of such assays indicated the samples were originated from healthy subjects. The samples were extracted by an affinity procedure adapted from Davies et al. This polyacrilamide gel presents affinity for cis-diol groups present in most of the studied nucleosides, except 2dA and T and high binding capacity for small molecules in basic medium. In acidic medium, however, the resin unbinds the cis-diol groups and analytes may be eluted. The SPE column was equilibrated with 20 mL of 0. An aliquot of 1 mL of the sample was added, followed by 4 mL of 0. Nucleosides were eluted by employing 4 mL of 0. Eluate was evaporated under nitrogen flow to half the volume and lyophilized for 24 h. The sample was ressuspended in 1 mL of deionized water and analyzed by CE-UV, showing the method sensitivity, since Validation All validation parameters were evaluated in standard solutions prepared in the biological matrix, i. All assays were performed in triplicate in the biological matrix, employing a pool composed by blood serum from 10 subjects spiked with standard solutions before extraction. The serum was centrifuged in ultrafiltration membranes and the filtrate was spiked with the desired amount of 1. The resulting solution was extracted as earlier described and injected. Results and Discussion Method development Aiming at the best separation of nucleoside standards, several electrophoretic parameters were evaluated. For assessment of the best conditions, electrophoretic mobility and resolution graphs against each evaluated parameter were constructed. Peaks were identified by UV absorption spectra and migration times. BGE pH and composition were first evaluated. A good compromise between separation and analysis time was observed for borate buffer at pH 9. BGE concentration was evaluated employing borate buffer solutions from 20 to mmol L-1 at pH 9. The best separation and a suitable analysis time were observed with 20 mmol L-1 borate buffer due to higher EOF velocity under more diluted BGEs Figure 2b. However, capillary zone electrophoresis CZE mode was not considered adequate for analytes separation since peak resolutions did not reach the electropherogram baseline. It was noticed that the resolution between peaks was improved with higher SDS concentrations. The best separation was obtained with 20 mmol L-1 borate buffer at pH 9. Nevertheless, under this condition, co-migration of C and 1mA, and 2dA and 8BrG internal standard pairs was observed Figure 2c , leading to the investigation of organic modifier addition to BGE. In fact, nucleosides present very similar structure, which differs slightly in charge to radius ratio. However, hydrophobicity differences are more prominent, justifying the best separations obtained with surfactant and organic modifier addition to the BGE. Voltage did not affect the separation, but the analysis time. For this reason, the value of 25 kV was applied in all subsequent analysis aiming at smaller analysis time and avoiding the Joule effect. The high surfactant concentration caused problems like capillary clogging and breakage, as well as formation of bubbles and salt deposition inside the equipment due to high SDS concentration. The system was not able to maintain a stable current throughout successive runs, therefore poor reproducibility was frequently observed. The capillaries needed to be changed often and instrument maintenance was necessary at least once a week. The overall procedure could take up to half a day and the constant changes of capillary represented an additional cost. Therefore, after method optimization, this parameter was reevaluated aiming at a better compromise between separation, analysis time and equipment maintenance reduction. SDS concentration was reinvestigated within the concentration range of to mmol L Electrophoretic mobilities did not vary significantly with lower SDS concentrations, but suitable resolutions and efficiencies were found with mmol L Thus, this concentration was employed in the subsequent analyses. Likewise, methanol concentrations were reinvestigated after SDS reevaluation on a fine tuning Figure 2e.

Figure 3 shows an electropherogram obtained under the optimized analysis conditions. Total analysis time was less than 25 min, the peak efficiencies were within a range of 4. Resolution values obtained by the optimized method are presented in Table 3.