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Determination of sarcosine as possible tumour marker of prostate tumours

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Abstract

Amino acid sarcosine, known also as N-methylglycine, may be established as new very important marker in prostate malignant tumours and may be determined by very simple test. Cancer of prostate is one of the most incident types of malignant tumours in men. More than one thousand men in Czech Republic die due to this disease. As well as in the case of other malignant tumours, for initiation of treatment well timed diagnosis of disease is necessary. For determination of sarcosine we employed the ionex chromatography with postcolumn derivatization by ninhydrin. We achieved the calibration curve linearity R^2=0.9984 with limit of detection 500nM. Moreover we confirmed that the calibration was not affected by presence of another aminoacids and ensure that selectivity of separation is near to 99% efficiency.

Keywords: sarcosine, new marker of prostate cancer, cysteine and homocysteine

Introduction

Sarcosine is naturally occurring non-toxic amino acid soluble in water. Sarcosine may be placed into group of biogenic amines, which occurs in different types of foods (fish, meat, beer, wine, cabbage, olives). In foods, sarcosine is originated during process of proteins fermentation by action of various kinds of organisms. At its consummation, sarcosine (Fig 1) passes to urine in unchanged form. Sarcosine also originates in liver and kidneys as intermediate of choline metabolism. At the beginning of 2009, study demonstrating diagnostic potential of this amino acid was published in very prestigious journal Nature. Sarcosine is very reputable marker also in early stages of tumours. Very important is also fact that sarcosine was not present in urine of healthy people. Due to this fact, probability of false positivity of investigation is minimized. In accordance with published results, sarcosine is significantly preferable marker of prostate cancer to prostate-specific antigene, whose presence in blood is routinely analyzed. Presence of amino acid sarcosine indicates rightly aggressive, high-malignant tumours. Aim of our work consisted in designation of chromatographic method for determination of sarcosine in presence of free amino acids as cysteine and homocysteine.

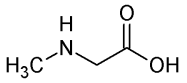

Figure 1: Structure of sarcosine

Materials and methods

Sarcosine of purity 99 % was obtained from Fluka BioChemika (Switzerland). Solution of sarcosine for preparing of calibration curve was prepared in buffer Na:TDG (N_3Na - 0.10 g, NaCl-11.5 g, $C_6H_8O_7$-14 g in 11 of H_20). Separation of sarcosine was carried out by the help of ionex chromatography on apparatus Amino Acid Analyzer AAA 400 (Ingos, Czech Republic). AAA analyzer works on basis of medium pressure liquid chromatography with ionex column and ninhydrin postcolumn derivatization. Glass column with inner diameter 3.7 mm and 350 mm length was filed manually with strong catex ionex in natrium cycle LG ANB with approximately 12µm particles and porosity 8%. Column was tempered in range 35 - 95°C. Double channel VIS detector with inner cell volume 5 µl worked statically under two wavelengths 440 and 570nm. Solution of ninhydrin (Ingos, Czech Republic) was prepared by dilution in 75 % v/v metylcelosolve (Ingos, Czech Republic) and in 25 % v/v 4M acetic buffer (pH5.5). For reduction $SnCl_2$ (Lachema, Czech Republic) was used. Prepared solution of ninhydrin was stored under inert atmosphere (N_2) in dark and cooled. Elution of Sarcosine was done by buffer contains Citric acid 11.11g, natrium citrate 4.04g and NaCl 9.25g x1L^{-1}. Flow rate was 0.3 ml/min. Reactor

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temperature was 120°C. Conductivity of used miliQ water was 18MΩ (Jamaspishvili et al. 2010).

Results and discussion

In experimental part, firstly we were focused on analytical determination of sarcosine. Ionex chromatography was chosen as the most suitable analytical technique. Sarcosine was analyzed in tested concentration range from 5 to 1000 μM with error of determination of about 8%. In chromatograms very well developed and separated chromatographic signals at retention time of 32 min were obtained. Calibration curve was linear in entire tested range with equation y=0.0266x-0.2794 R^2=0.9984 (Fig 2). Limit of detection of sarcosine was determined as 3S/N and was about 500 nM. Limit of quantification of sarcosine was determined as 10 S/N - 5 μM.

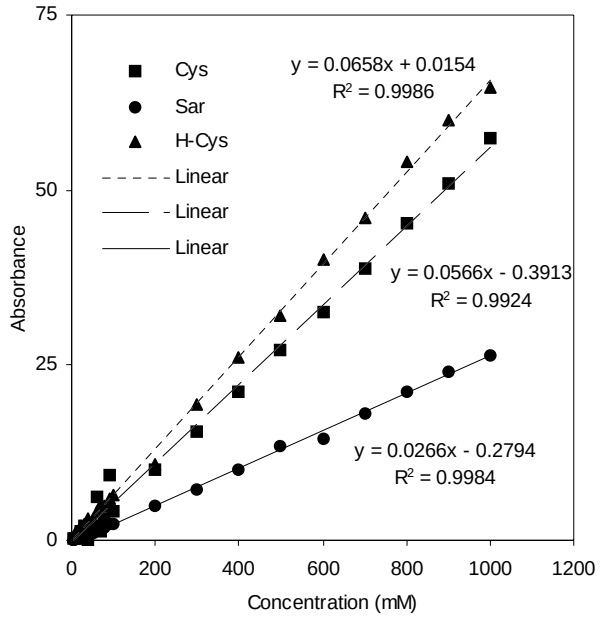

Figure 2: Calibration curve of sarcosine in presence of cysteine and homocysteine

Analysis of sarcosine in presence of two very hardly determinable amino acids (cysteine and homocysteine) was carried out in subsequent experiments. Presence of these two amino acids had no effect on chromatographic signals of sarcosine. All chromatographic signals were well developed and separated. Moreower free amino acids are present in biological samples – due to this fact it is absolutely necessary to propose suitable technique for determination of sarcosine in presence of free amino acids. In our next experiment, sarcosine at concentration 10 μM was added into mixture of 17 amino acids of concentration 20 μM. In Fig 3, typical chromatographic record is demonstrated. Individual amino acids are separated very well; no interferences with determined sarcosine were observable.

Conclusion

Chromatographic method enabling simultaneous determination of sarcosine in presence of 17 free amino acids was designed in our experimental work.

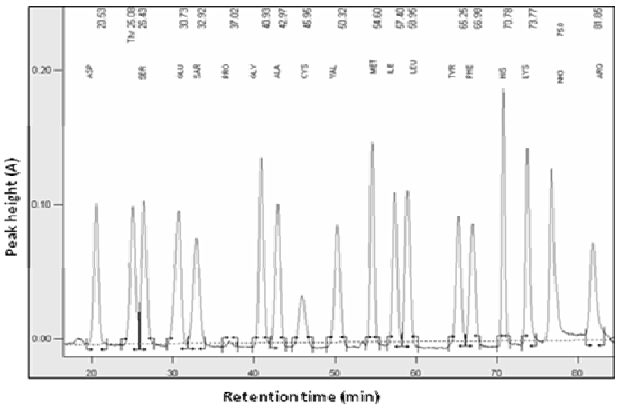

Figure 3: Typical chromatographic record of common amino acids with addition of sarcosine

Acknowledgements

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