ASSESSING OF COMPLEXITY AND RELIABILITY OF URINARY NUCLEOSIDES PATTERNS BY THEIR METABONOMIC ANALYSIS

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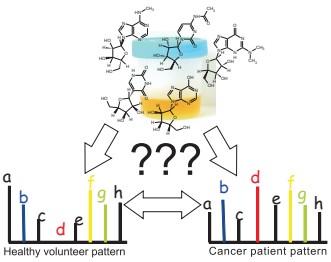
INTRODUCTION

Nucleosides as the degradation products of nucleic acids are one particular class of metabolites present in human urine, that has been of interest of clinical and biomedical studies. They have been investigated as universal tumour biomarkers i.e. untargeted diagnostic and prognostic tool of cancer diseases.
However, still their clinical significance is unknown due to high complexity of nucleosides patterns containing above 80 different compounds and conducted studies limited to a small group of subjects, what limit their informational content.



Metabonomics could be a convinient approach to study nucleosides patterns. Thanks to applied analytical and chemometric tools and numerous samples and compounds considered it enables a powerful and comprehensive analysis of nucleosides patterns complexity. Moreover, during metabonomics studies reliability of relationship between nucleoside profile and cancer disease could be verified and evaluated.

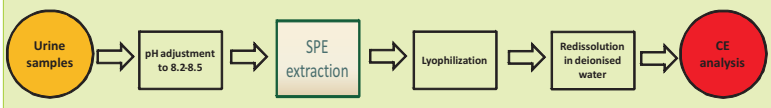
PARTICIPANTS AND SAMPLE COLLECTION

86 healthy and 72 cancer subjects with diagnosed bladder, kidney, testis or prostate tumour volunteered to participate in the studies. Patients history including disease stage and applied therapy was taken from all urogenitial tract cancer participants.
Each individual provided first-morning urine sample and lifestyle survey.

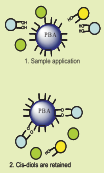
AIM OF STUDY

Investigation of relationship between urinary nucleosides pattern and cancer disease by means of capillary electrophoresis analysis of urine samples and exploration and interpretation of obtained profiles by chemometric tools.

ANALYTICAL PROCEDURE



Solid phase extraction



In solid phase extraction due to specific affinity of used phenylboronate gel (PBA) to cis-diol groups, which are present in structure in nucleosides, selectivity of extraction process was achieved.

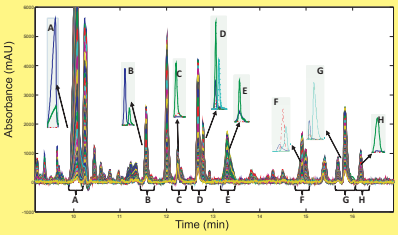
Capillary electrophoretic analysis

CE method parameters: fused silica capillary, 50 µm I.D., 70 cm effective length, 80 cm total length, BGE: 100/72/150 mM borate/phosphate/SDS; pH 6.7; 25 kV, 30°C, 5 s injection at 0.5 p.s.i.; PDA detection (quantification at 254 nm).

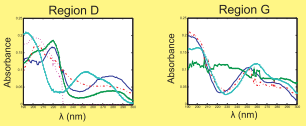
CE method was succesfully validated and used in analysis of nucleosides patterns from urine samples.

MULTIVARIATE CURVE RESOLUTION RESULTS

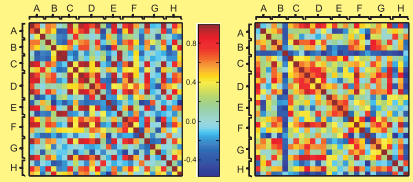
DATA: 8 regions of multiwavelength electropherograms collected with help of PDA detector, resolved with multivariate curve resolution method giving concentration profiles and spectra of 28 different metabolites. Multivariate curve resolution-alternating least squares (MCR-ALS) and matrix augmentation of analyzed regions was employed.

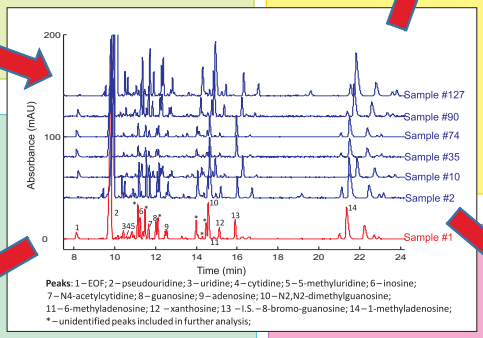


Spectra obtained for region D and G



Correlation matrices for group of healthy and cancer patients was created on the basis of Pearson correlation coefficient calculated for each pair of metabolites.





Peaks: 1 – EOF; 2 – pseudouridine; 3 – uridine; 4 – cytidine; 5 – 5-methyluridine; 6 – inosine; 7 – N4-acetylcytidine; 8 – guanosine; 9 – adenosine; 10 – N2,N2-dimethylguanosine; 11 – 6-methyladenosine; 12 – xanthosine; 13 – I.S. – 8-bromo-guanosine; 14 – 1-methyladenosine; * – unidentified peaks included in further analysis;

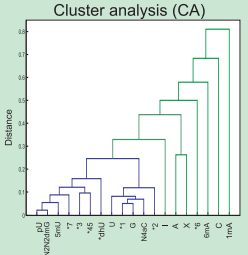
LEVELS OF SELECTED NUCLEOSIDES

DATA: Levels of 19 nucleosides normalized by creatinine level, calculated on the basis of peak areas and appropriate calibration curves equations.

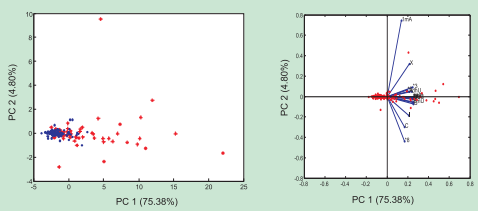
Univariate analysis by U Mann Whitney test (α=0.01)

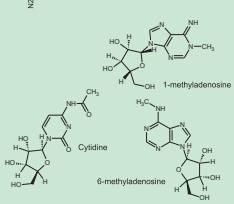
Metabolite	Healthy voluntaers(n = 85)		Cancer patients (n = 64)		Elevated value(%)	p value	Significance
	Range	Mean±SD	Range	Mean±SD			
pU	13.67 – 81.67	38.07 ± 13.13	27.91 – 336.85	78.74 ± 57.41	42.19	0.0000	<0.0001
U	0.23 – 1.31	0.66 ± 0.22	0.41 – 6.31	1.45 ± 1.13	48.44	0.0000	<0.0001
C	0.05 – 0.79	0.24 ± 0.16	0.06 – 2.91	0.40 ± 0.42	23.44	0.0044	<0.005
5mU	0.20 – 1.69	0.74 ± 0.26	0.44 – 7.50	1.63 ± 1.32	46.88	0.0000	<0.0001
*1	0.69 – 6.47	2.64 ± 1.09	1.15 – 31.20	5.38 ± 4.51	39.06	0.0000	<0.0001
I	0.09 – 0.99	0.32 ± 0.17	0.20 – 2.34	0.72 ± 0.50	43.75	0.0000	<0.0001
*2	0.48 – 10.40	1.61 ± 1.10	1.14 – 16.70	3.52 ± 2.76	32.81	0.0000	<0.0001
NAaC	0.15 – 1.89	0.87 ± 0.33	0.44 – 7.37	1.73 ± 1.26	42.19	0.0000	<0.0001
G	0.31 – 1.67	0.81 ± 0.31	0.50 – 8.81	1.80 ± 1.46	42.19	0.0000	<0.0001
*3	0.09 – 2.93	1.10 ± 0.47	0.49 – 7.07	2.03 ± 1.44	34.38	0.0000	<0.0001
A	0.06 – 1.14	0.47 ± 0.21	0.15 – 2.98	0.74 ± 0.51	20.31	0.0001	<0.0001
*4S	0.64 – 3.44	1.56 ± 0.58	0.98 – 11.67	3.20 ± 2.40	40.63	0.0000	<0.0001
*6	0.10 – 0.95	0.33 ± 0.16	0.01 – 3.06	0.63 ± 0.62	28.13	0.0002	<0.0005
*7	0.18 – 2.33	0.64 ± 0.29	0.16 – 4.90	1.26 ± 0.87	39.06	0.0000	<0.0001
N2N2dmG	0.93 – 5.70	2.61 ± 1.00	0.58 – 23.22	5.44 ± 4.25	39.06	0.0000	<0.0001
6mA	0.08 – 2.06	0.43 ± 0.25	0.04 – 4.33	0.87 ± 0.73	32.81	0.0000	<0.0001
X	0.48 – 6.31	1.36 ± 0.83	0.81 – 10.51	2.81 ± 1.86	34.38	0.0000	<0.0001
1mA	0.65 – 4.92	2.18 ± 0.80	1.33 – 45.70	4.40 ± 5.75	35.94	0.0000	<0.0001
*dhU	2.36 – 30.64	9.23 ± 3.93	4.14 – 71.17	17.94 ± 13.35	34.38	0.0000	<0.0001

Cluster analysis (CA)



Principal Component Analysis (PCA)

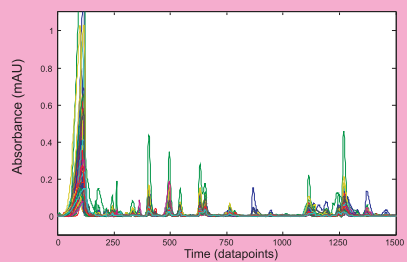




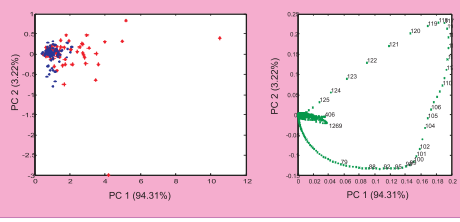
ELECTROPHORETIC FINGERPRINTS

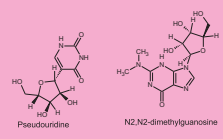
DATA: Electrophoregrams collected during CE analysis of urine samples after preprocessing.

Preprocessing included: baseline correction,denoising (wavelet transform) and peak shift correction by correlation optimized warping (COW)



Principal Component Analysis (PCA)





CONCLUSIONS

1. Metabonomic approach comprising developed analytical procedure and appropriate chemometric tools could be an effective tool of urinary nucleosides studies
2. Different types of metabonomic data could be used in evaluation of relationship between urinary nucleosides patterns and cancer disease leading to resembling statements
3. Relationship between urinary nucleosides pattern and presence of cancer was demonstrated. It was expressed by elevated levels of most nucleosides and differences in metabolite-metabolite relations.

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2. Szymańska E., Markuszewski M. J., Capron X., Nederkassel A.-M., Vander Heyden Y., Markuszewski M., Krajka K., Kaliszan R., Electrophoresis, 2007, 28: 2861-2873.
3. Szymańska E., Markuszewski M.J., Bodzioch K., Kaliszan R., J. Pharm. Biomed. Anal., 2007,44: 1118-1126.