Targeted Quantitative Metabolomics Using Dried Plasma Spots Cards

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BIOCRATES LIFE SCIENCES The Deep Phenotyping Company

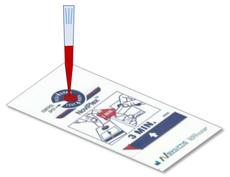
Introduction

- The dried plasma spot (DPS) cards (Noviplex™, Novilytic Labs), a novel miniturarized blood fractionation technology, enables collection of a 2.5 µL of plasma
- DPS cards eliminates the need for venipuncture and specialized blood collection tubes commonly used to obtained plasma samples.
- DPS is especially attractive for the use in clinical metabolomics research.
- In this work, a quantitative metabolite profile was obtained for the first time by dried plasma spot cards and comprehensively compared to the established EDTA-plasma methodology
- The targeted quantitative metabolite analysis was carried out employing Absolute*IDQ*® p180 Kit (Biocrates Life Sciences)

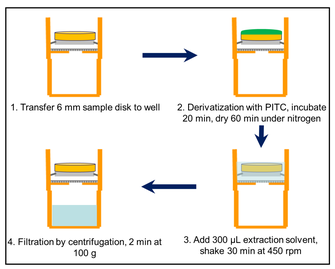
Materials and methods

Noviplex™ dried plasma spot cards

- Volumetric plasma sample collection
  - Plasma sampling volumes of 2.5 µL
  - Independent of hematocrit
- 20 to 75 µL of whole blood is required
  - Finger-stick
  - Mouse tail-bleed
- Rapid sample preparation for MS-based analysis



- **50 µL of EDTA whole blood** sample was pipetted on to the labelled test area
- It was verified that control spot changed red



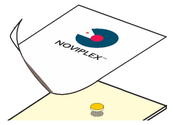
Sample preparation according to Absolute*IDQ*® p180 Kit User Manual

Sample extract dilution
- 1:5 for FIA-MS/MS
- 1:2 for LC-MS/MS

Analysis carried out on AB Sciex 4000 QTRAP® MS/MS system coupled to Agilent 1200 HPLC pump

Biocrates Absolute*IDQ*® p180 Kit

- 10 µL of sample (plasma/serum)
- Quantification up to 188 metabolites
  - LC-MS/MS and FIA-MS/MS analysis
- Isotope-labeled and chemically homologous internal standards are used for quantification
- 3 hours sample preparation
  - Standardized assay in 96-well plate format
- Validated for several MS/MS platforms



- The top layer of the test card was peeled off after 3 min
- Cards were **dried for 15** min at room temperature
- Sample disk was removed for metabolomics analysis

Absolute*IDQ* p180 Kit

Met*IDQ* software

Data evaluation and data processing for FIA-MS/MS

Results

Table 1. Concentrations of quantified metabolites in DPS samples

Amino Acids									
Ala	504 ± 56	Gln	532 ± 90	Ile	64.4 ± 9	Orn	19.9 ± 4.4	Thr	141 ± 19
Arg	41.2 ± 9	Glu	18 ± 2	Leu	113.6 ± 14.4	Phe	74.4 ± 7.7	Trp	46.4 ± 4.2
Asn	44 ± 7	Gly	214 ± 35	Lys	36.3 ± 6.3	Pro	313 ± 41	Tyr	92 ± 10.1
Cit	24 ± 5	His	64.4 ± 5.3	Met	32 ± 3.4	Ser	80 ± 12	Val	252.4 ± 34.1
Biogenic Amines					**Acylcarnitines**			**Sugars**	
Creatinine	87.2 ± 6.8	Spermidine	0.5 ± 0.1	C0	40 ± 4	C18:1	0.25 ± 0.03	Hexose	4972 ± 517
Kynurenine	1.9 ± 0.3	Taurine	34.8 ± 6	C3	0.4 ± 0	C18:2	0.15 ± 0.04		
t4-OH-Pro	5.8 ± 1.2			C2	4.84 ± 0.47				
Phosphatidylcholines									
lysoPC a C16:0	69.6 ± 6.9	PC aa C34:1	184 ± 17	PC aa C38:6	69.2 ± 6	PC ae C32:2	0.93 ± 0.11	PC ae C38:5	13.3 ± 1.2
lysoPC a C16:1	2.7 ± 0.3	PC aa C34:2	310 ± 28	PC aa C40:1	1.6 ± 0.2	PC ae C34:0	1.97 ± 0.21	PC ae C38:6	6.1 ± 0.7
lysoPC a C17:0	2.2 ± 0.2	PC aa C34:3	15.3 ± 1.4	PC aa C40:2	2.1 ± 0.3	PC ae C34:1	11.2 ± 1.2	PC ae C40:1	2.6 ± 0.25
lysoPC a C18:0	18.6 ± 1.9	PC aa C34:4	1.5 ± 0.2	PC aa C40:3	1.9 ± 0.2	PC ae C34:2	10.2 ± 1	PC ae C40:2	4.2 ± 0.4
lysoPC a C18:1	15.4 ± 1.4	PC aa C36:0	7.1 ± 0.9	PC aa C40:4	3.9 ± 0.4	PC ae C34:3	7.8 ± 0.71	PC ae C40:3	2.7 ± 0.3
lysoPC a C18:2	26.4 ± 2	PC aa C36:1	47.2 ± 5.4	PC aa C40:5	6.8 ± 0.7	PC ae C36:0	1.39 ± 0.22	PC ae C40:4	3.8 ± 0.4
lysoPC a C20:3	1.7 ± 0.2	PC aa C36:2	188 ± 20	PC aa C40:6	21.9 ± 2.2	PC ae C36:1	10.2 ± 0.9	PC ae C40:5	4.6 ± 0.4
lysoPC a C20:4	5 ± 0.5	PC aa C36:3	86 ± 9	PC aa C42:0	1.06 ± 0.13	PC ae C36:2	17.3 ± 1.7	PC ae C40:6	5.3 ± 0.5
lysoPC a C24:0	1.9 ± 0.3	PC aa C36:4	131 ± 12	PC aa C42:1	0.72 ± 0.08	PC ae C36:3	6.28 ± 0.61	PC ae C42:1	1.3 ± 0.1
lysoPC a C26:0	2.4 ± 0.3	PC aa C36:5	16 ± 1.5	PC aa C42:2	0.93 ± 0.11	PC ae C36:4	13.8 ± 1.3	PC ae C42:2	1.7 ± 0.3
lysoPC a C28:0	2.0± 0.1	PC aa C36:6	0.92 ± 0.14	PC aa C42:4	0.77 ± 0.07	PC ae C36:5	8.76 ± 0.89	PC ae C42:3	1.7 ± 0.2
lysoPC a C28:1	1.7 ± 0.1	PC aa C38:0	5.7 ± 0.7	PC aa C42:5	0.62 ± 0.08	PC ae C38:0	2.9 ± 0.22	PC ae C42:4	1.7 ± 0.2
PC aa C24:0	0.97 ± 0.13	PC aa C38:1	5.3 ± 0.8	PC aa C42:6	0.82 ± 0.09	PC ae C38:1	2.91 ± 0.42	PC ae C44:3	0.5 ± 0.1
PC aa C32:0	13.3 ± 1.4	PC aa C38:3	27.8 ± 2.9	PC ae C30:0	1.02 ± 0.11	PC ae C38:2	5.32 ± 0.69	PC ae C44:4	0.85 ± 0.08
PC aa C32:1	13.7 ± 1.4	PC aa C38:4	66.4 ± 6.9	PC ae C30:2	0.68 ± 0.08	PC ae C38:3	5.76 ± 0.66	PC ae C44:5	2.2 ± 0.3
PC aa C32:2	4.04 ± 0.45	PC aa C38:5	37.7 ± 3.3	PC ae C32:1	3.1 ± 0.32	PC ae C38:4	12.6 ± 1	PC ae C44:6	1.4 ± 0.1
Sphingomyelins									
SM (OH) C14:1	10.4 ± 1	SM (OH) C22:2	18 ± 1.9	SM C16:0	146 ± 14	SM C18:0	31 ± 2.8	SM C24:1	30.6 ± 3.5
SM (OH) C16:1	4.9 ± 0.5	SM (OH) C24:1	1.6 ± 0.2	SM C16:1	18.2 ± 1.7	SM C18:1	13.4 ± 1.6	SM C26:1	0.64 ± 0.36
SM (OH) C22:1	19.2 ± 2.1								

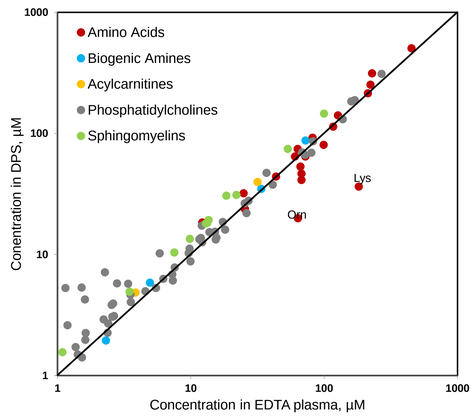


***Fig.1**: Correlation between metabolite concetrations in DPS and EDTA-plasma samples from the same individual*

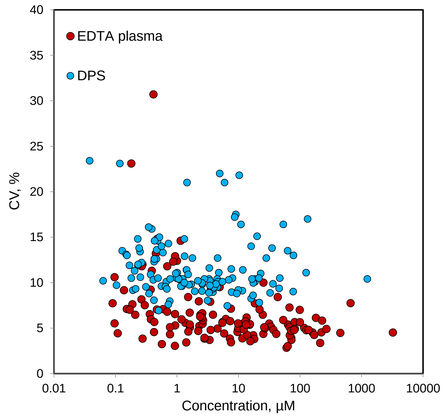


***Fig.2**: Distibution of CVs of repeated measurements (n=8) for DPS and EDTA-plasma samples*

Conclusions

- The quantitative results for 127 endogenous metabolites in human blood samples using DPS cards were obtained.
- The analytical figures of merit for DPS and EDTA-plasma sampling methodologies were in a good agreement
- The data transferability and comparability with the standard approach (EDTA-plasma) is metabolite dependent.
- The DPS is ready for routine applications in targeted quantitative metabolomics
- The DPS is excellent sampling device for standardized comprehensive metabolome analysis with several advantages:
  - Very low sample volume (e.g. for translational longitudinal studies in mice and men)
  - Less invasive
  - Easy sample transport/shipping

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