Supplementary information

New insights into the conversion of electropherograms to the effective electrophoretic mobility scale

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A Materials and methods

A.1 Chemicals and reagents

Acetic acid and formic acid were purchased from Biosolve (Dieuze, France). Water, methanol, isopropanol (iPrOH), and acetonitrile (ACN) were purchased from Fisher Scientific (Loughborough, United Kingdom). Standard compounds were purchased from Sigma Aldrich (Buchs, Switzerland).

A.2 Standard solution preparation

Individual stock solutions of compounds were prepared in 5% v/v ACN and 0.1% v/v FA at a final concentration of 1 mg/mL and stored at -80°C. Mix stock solutions were prepared in 5% v/v ACN and 0.1% v/v FA at 10 µg/mL and stored at -80°C. Mix stock was extemporaneously diluted to 500, 250, 125 and 62.5 ng/mL with water.

A.3 Cell culture preparation

Four replicates of 2D-cell cultures of astrocytes were grown in the presence of different natural neuro-inflammatory triggers at different concentrations, namely interleukin 1β (IL-1β) at 30 ng/mL, tumor necrosis factor α (TNFα) at 30 ng/mL, and lipopolysaccharide (LPS) at 10 µg/mL. The control cell culture was grown in parallel in absence of any inflammatory trigger. After two weeks of growth, cell cultures were snap-frozen in liquid nitrogen. Protein precipitation was achieved by adding 1 mL of a cold solution (-20°C) of MeOH:H2O (80:20 v/v), scraping, and by vortexing during 1 minute. Samples were centrifuged at 14000 g during 15 minutes at 4°C, the supernatant was collected and then evaporated to dryness before resuspension in 100 µL of a solution made of MeCN:H2O (50:50 v/v). Quality control (QC) samples were prepared by pooling the same volume from each sample after reconstitution. Volumes of 10 µL of individual cell culture extracts, QC's and diluted QC's were evaporated to dryness using a SpeedVac (ThermoFisher, Langenselbold, Germany). Before injection, samples were reconstituted with 10 µL of an aqueous solution containing paracetamol, procaine and ethyl-sulfate at a concentration of 50, 5 and 5 µg/mL respectively.

A.4 BGE and sheath-liquid preparation

Through the study, 10% v/v acetic acid in water was used as BGE. Sheath-liquid was composed of isopropanol-water-acetic acid (50:50:1 v/v/v) in the validation part. In order to
enlarge metabolome coverage by facilitating the ionization of the different metabolites under ESI+ and ESI- conditions, in the metabolomics profiling part, the concentration of acetic acid in the sheath liquid was reduced to 5 mM. Purine and HP-0921 were purchased from Agilent technologies (Santa Clara, CA, USA, P/N: G1069-8001) and used as lock masses for the Q-ToF instrument after being spiked into the sheath-liquid to yield final concentrations of 50 and 25 nM, respectively.

A.5 Validation analyses

A triple quadrupole platform was used for the study and validation of the formulas derived in the theory section. The separation was carried out with a G7100 capillary electrophoresis (CE) system from Agilent Technologies (Waldbronn, Germany). Separations were performed using a fused silica capillary purchased from BGB technologies (Boeckten, Switzerland) with a total length of 70 cm and an internal diameter of 50 µm. Prior to its first use, the capillary was conditioned with MeOH, H₂O, NaOH 1M, H₂O, HCl 1M, H₂O, HCl 0.1M, H₂O, and BGE at 5 bar during 1 minute each. Injections were performed hydrodynamically by application of 50 mbar during 12 s, using ~1% of the capillary total length, circa 14 nL. Injected volumes were calculated with Zeecalc v1.0b (https://ispso.unige.ch/labs/fanal/zeecalculator). Separation was performed by application of +30 kV for positive mode ESI ionization, or at –30 kV for negative ESI. Before each analysis, the capillary was washed with MeOH and BGE at 5 bar during 1 minute. To avoid temperature inhomogeneities between the capillary parts inside and outside the CE instrument, the CE thermostat was set at room temperature (~23 °C).

The CE system was hyphenated with an Agilent 6490 triple quadrupole mass spectrometer (QqQ MS, Agilent Technologies, Santa Clara, CA, US) equipped with an ESI source via a coaxial sheath-flow interface with a standard triple-tube sprayer (P/N G1607B) from Agilent Technologies. The sheath liquid was delivered at a flow rate of 3 µL/min, using a 2300 Series isocratic pump purchased from Agilent Technologies (Waldbronn, Germany) equipped with a 1:100 split. Electrospray ionization was operated in positive mode, and spectra were acquired via SRM measurements. The pressures and injection volumes used during the validation are described in section 4.2. The precursor and productions monitored for each compound and the collision energies are reported in Table C in the supplementary information. The following source parameters were used: the nebulizing gas pressure was set at 0 psi and the sheath gas at 11 L/min and 150°C. The capillary voltage was adjusted to 5500 V. The ion funnel voltages were set at 150 V for the high-pressure funnel and 60 V for the low-pressure one. The EMV voltage was set at 400 V and the cell accelerator voltage at 5 V. For all transitions, precursor and product ion selection was performed with a resolution of 1.2 and 0.7 m/z, respectively. Data acquisition and instrument control were performed using MassHunter version B.08.00 (Agilent, Santa Clara, US).

A.6 Untargeted metabolomics

The CE setup for the untargeted metabolomics profiling was the same as described in section A.5 for the validation. The CE system was in turn hyphenated to a maXis-3G QTOF MS from Bruker (Bremen, Germany), equipped with an ESI source via a coaxial sheath-flow ESI
interface with a standard triple-tube sprayer (Agilent P/N G1607A) and a platinum needle. The sheath liquid was delivered at a flow rate of 3 µL/min, using a 2300 Series isocratic pump purchased from Agilent Technologies (Waldbronn, Germany) equipped with a 1:100 split.

For cationic profiling, ESI was operated in positive mode with the following MS parameters: nebulizer and sheath gas were set to 0 bar and 10 L/min, 100°C, respectively. Capillary, end-plate and funnel voltages were respectively adjusted to 6000, 400 and 300 V. For anionic profiling, ESI was performed in negative mode with the following source parameters: nebulizing gas and sheath gas were set to 0.3 bar and 4 L/min, 150°C respectively. Capillary, end-plate and funnel voltages were adjusted to 4000, 400 and 300 V, respectively. MS acquisitions were performed at a frequency of 1 Hz, with a mass range going from 50 to 1000 m/z.
## B Table of standard compound mobilities

ROMANCE v1 values taken from [7]. ROMANCE v2 values taken from 2-marker formula results in section 4.2.

| Compound           | Mobility ROMANCE V1 (mm² kV⁻¹ min⁻¹) | Mobility ROMANCE V2 (mm² kV⁻¹ min⁻¹) |
|--------------------|---------------------------------------|---------------------------------------|
| Acetylcholine      | 1913                                  | 1879                                  |
| Adenine            | 1865                                  | 1795                                  |
| Choline            | 2184                                  | 2175                                  |
| L-histidine        | 1760                                  | 1747                                  |
| L-lysine           | 1941                                  | 1881                                  |
| Nicotinamide       | 1830                                  | 1773                                  |
| Phenethylamine     | 1839                                  | 1824                                  |
| Thiamine           | 2333                                  | 2321                                  |
| Tryptamine         | 1669                                  | 1616                                  |
| Tyramine           | 1626                                  | 1626                                  |
| 3-methoxytyramine  | N/A                                   | 1411                                  |
| Agmatine           | N/A                                   | 3119                                  |
| Amphetamine        | N/A                                   | 1598                                  |
| GABA               | N/A                                   | 1774                                  |
| L-glutamine        | N/A                                   | 1833                                  |
## C Monitored transitions for the reference compounds

| Compound            | Precursor ion (m/z) | Product ion (m/z) | Collision energy (V) | Dwell time (ms) |
|---------------------|---------------------|-------------------|----------------------|-----------------|
| Acetylcholine       | 146.1               | 87.2              | 13                   | 14              |
| Adenine             | 136.2               | 118.9             | 25                   | 14              |
| Choline             | 104.1               | 60.1              | 17                   | 14              |
| L-histidine         | 156.2               | 110.0             | 13                   | 14              |
| L-lysine            | 147.1               | 84.1              | 17                   | 14              |
| Nicotinamide        | 123.1               | 80.1              | 17                   | 14              |
| Phenethylamine      | 122.1               | 105.1             | 9                    | 14              |
| Thiamine            | 265.1               | 122.0             | 13                   | 14              |
| Tryptamine          | 161.1               | 144.2             | 9                    | 14              |
| Tyramine            | 138.1               | 121.1             | 9                    | 14              |
| 3-methoxytyramine   | 168.1               | 151.2             | 9                    | 14              |
| Agmatine            | 131.1               | 60.1              | 9                    | 14              |
| Amphetamine         | 136.1               | 91.0              | 17                   | 14              |
| GABA                | 104.2               | 87.0              | 9                    | 14              |
| L-glutamine         | 147.1               | 84.1              | 17                   | 14              |
## D Tables of PCA loadings

List of identified metabolites and mobility-based PCA loadings (see Figure 4) from the untargeted metabolomics analysis (on astrocyte inflammation).

### D.1 ESI– mode

| Metabolites                        | Component 1 | Component 2 |
|------------------------------------|-------------|-------------|
| D-pantothenic acid                 | -0.08       | +0.46       |
| 5-oxo-D-proline                    | -0.24       | -0.33       |
| N-acetyl-DL-serine                 | +0.18       | -0.33       |
| N-acetyleneuraminic acid           | -0.17       | -0.31       |
| SN-glycerol 3-phosphate            | -0.20       | +0.27       |
| Gluconic acid                      | +0.01       | -0.33       |
| 6-phosphogluconic acid             | +0.29       | -0.14       |
| N-acetyl-L-aspartic acid           | -0.31       | -0.07       |
| Dihydroxyacetone phosphate         | +0.30       | +0.11       |
| CDP-ethanolamine                   | +0.18       | +0.24       |
| D-glucose 6-phosphate              | +0.29       | +0.02       |
| Fructose 1,6-biphosphate           | +0.29       | -0.06       |
| (S)-(-)-L-malic acid               | +0.27       | +0.04       |
| 3-methyl-2-oxovaleric acid         | -0.17       | -0.19       |
| Citric acid                        | +0.21       | -0.14       |
| D-ribose 5-phosphate               | +0.23       | -0.07       |
| Guanosine 5’-diphosphate           | -0.07       | -0.20       |
| D-(+)-galactose                    | -0.14       | +0.12       |
| Creatine phosphate                 | +0.15       | +0.12       |
| (R,R)-L-(+)-tartaric acid          | +0.16       | -0.07       |
| 3-(4-hydroxyphenyl)lactic acid     | -0.15       | +0.05       |
| Phenylacetic acid                  | +0.05       | -0.15       |
| Adenosine 3’,5’-diphosphate         | -0.11       | -0.10       |
| Cytidine 2’,3’-cyclic mono-phosphate| +0.15       | -0.04       |
| Adenosine 5’-monophosphate          | +0.13       | -0.04       |
| Uridine 5’-diphosphate             | +0.10       | -0.08       |
| N-acetyl-DL-glutamic acid          | -0.01       | -0.10       |
| Uridine-5-monophosphate            | +0.06       | -0.03       |
### D.2 ESI+ mode

| Metabolites                        | Component 1 | Component 2 |
|------------------------------------|-------------|-------------|
| 5'-deoxyadenosine                  | +0.22       | -0.33       |
| Alpha-aminoadipic acid             | +0.16       | +0.35       |
| L-tyrosine                         | -0.31       | -0.12       |
| Thiamine pyrophosphate             | +0.05       | -0.33       |
| L-anserine                         | +0.30       | -0.12       |
| L-histidine                        | -0.31       | -0.08       |
| L-asparagine                       | -0.23       | +0.21       |
| Folic acid                         | -0.28       | -0.02       |
| D-mannosamine                      | +0.15       | +0.24       |
| Neopterin                          | +0.27       | -0.02       |
| Carnosine                          | +0.25       | -0.09       |
| L-phenylalanine                    | +0.16       | +0.21       |
| L-tryptophan                       | -0.24       | -0.11       |
| L-arginine                         | -0.06       | +0.26       |
| L-lysine                           | -0.06       | +0.24       |
| N,N-dimethylarginine               | -0.06       | +0.23       |
| O-acetyl-L-carnitine               | +0.21       | -0.08       |
| Bioppterin                         | +0.18       | -0.14       |
| Nicotinic acid                     | -0.13       | -0.18       |
| Citrulline                         | +0.03       | +0.22       |
| 5'-methylthioadenosine             | +0.21       | -0.05       |
| D-(+)-galactosamine                | +0.06       | +0.20       |
| S-(5'-adenosyl)-L-homocysteine     | -0.02       | -0.18       |
| N-alpha-acetyl-L-lysine            | -0.15       | -0.08       |
| Glutathione                        | +0.16       | -0.02       |
| Spermidine                         | +0.04       | +0.15       |
| Adenosine                          | -0.04       | -0.15       |
| N-amidino-L-aspartic acid          | -0.13       | +0.01       |
| Theobromine                        | -0.07       | -0.11       |
| Deoxycarnitine                     | +0.13       | +0.01       |
| Cytidine                           | -0.02       | -0.12       |
| Pyridoxal                          | -0.09       | -0.00       |
| Dopamine                           | +0.02       | -0.08       |
| Tyramine                           | -0.07       | -0.04       |
| Thiamine                           | -0.03       | -0.07       |
| Pyridoxamine                       | -0.05       | -0.03       |
| L-kynurenine                       | -0.03       | -0.04       |
| Isobutyryl-L-carnitine             | +0.00       | +0.02       |
E Detailed derivations

E.1 Analyte motion under a ramped field

The speed $v$ of an analyte in CE can be decomposed as

$$v = v_{EOF} + \mu E$$  \hspace{1cm} (17)

where $\mu$ is the effective electrophoretic mobility, $E$ is the applied field, and $v_{EOF}$ the speed of the flow. We further split this as

$$v_{EOF} = v_p + \mu_{EOF} E,$$  \hspace{1cm} (18)

where $\mu_{EOF}$ is the electroosmotic mobility and $v_p$ a constant term, which models the speed due to a pressure gradient.

Consider an electric field with a ramp-up phase during a time $t_R,$

$$E = \begin{cases} S_R \left( \frac{t}{t_R} \right) E_{\text{max}} & \text{if } 0 \leq t \leq t_R, \\ E_{\text{max}} & \text{if } t > t_R, \end{cases}$$  \hspace{1cm} (19)

where the shape function $S_R$ goes between $S_R(0) = 0$ and $S_R(1) = 1$. The analyte must travel a distance $L$, which is the integral of speed over time

$$L = \int_0^t v \, dt = \int_0^{t_R} \left( v_p + (\mu_{EOF} + \mu) E_{\text{max}} S_R \left( \frac{t}{t_R} \right) \right) \, dt + \int_{t_R}^t (v_p + (\mu_{EOF} + \mu) E_{\text{max}}) \, dt = (v_p + (\mu_{EOF} + \mu) E_{\text{max}}) t - \lambda (\mu_{EOF} + \mu) E_{\text{max}} t_R,$$  \hspace{1cm} (20)

assuming that $t > t_R$, as it is the case in usual conditions. We define

$$\lambda = 1 - \int_0^1 S_R(\tau) \, d\tau,$$  \hspace{1cm} (21)

and solving for $\mu$,

$$\mu = \frac{L}{E_{\text{max}}} \left( 1 - \frac{t_R v_p}{t - \lambda t_R} - \frac{v_p}{E_{\text{max}}} - \mu_{EOF} \right).$$  \hspace{1cm} (22)

By measuring the migration time $t_{\text{marker}}$ of a substance of known mobility $\mu_{\text{marker}}$ (for instance, the EOF with $\mu = 0$) we arrive at

$$\mu = \mu_{\text{marker}} + \frac{L}{E_{\text{max}}} \left( 1 - \frac{\lambda t_R v_p}{L} \right) \left( \frac{1}{t - \lambda t_R} - \frac{1}{t_{\text{marker}} - \lambda t_R} \right).$$  \hspace{1cm} (23)

The appearance of $v_p$ is due to the non-proportional effects that the constant part of the speed ($v_p$ itself) and the electric-field induced speed have on the analyte during the ramp phase. Consequently, this reduces to the usual formula when there is no ramp, $t_R = 0$. But even if a ramp is present, it can already be used for scale transformation if $v_p$ is zero, i.e., there is no pressure-induced speed.

VIII
If \( v_p \) is not known, we can take a second marker \((t_A, \mu_A)\) and find

\[
\mu = \frac{\mu_{\text{marker}} - \mu_A}{t_A - \frac{1}{t_{\text{marker}} - \mu_R}} (t - \lambda t_R) \left[ \frac{1}{t_{\text{marker}} - t_A} \right] + \frac{\left( t_{\text{marker}} - \lambda t_R \right) \mu_{\text{marker}} - (t_A - \lambda t_R) \mu_A}{t_{\text{marker}} - t_A} = \frac{(t - t_A) \left( t_{\text{marker}} - \lambda t_R \right) \mu_{\text{marker}} - (t - t_{\text{marker}}) (t_A - \lambda t_R) \mu_A}{(t_{\text{marker}} - t_A) (t - \lambda t_R)}. \tag{24}
\]

Notice that we no longer need to specify \( L \) or \( E_{\text{max}} \). It simplifies considerably if one of the two markers is the EOF, say \( \mu_{\text{marker}} = 0 \),

\[
\mu = \frac{(t - t_{\text{EOF}}) (t_A - \lambda t_R)}{(t_A - t_{\text{EOF}}) (t - \lambda t_R)} \mu_A. \tag{25}
\]

### E.2 Peak area transformations

Let us name the intensity profile by the function \( I(t) \). Then, the area under the curve of a peak in the electropherogram is given by

\[
A = \int I(t) \, dt. \tag{26}
\]

Changing variables from \( t \mapsto \mu(t) \), the integration measure changes by

\[
dt = \left| \frac{\partial \mu}{\partial t} \right| \, d\mu \implies A = \int I(\mu) \cdot \left| \frac{\partial \mu}{\partial t} \right|^{-1} \, d\mu. \tag{27}
\]

This has a simple expression as a function of \( t \), which can be obtained from (24),

\[
\frac{\partial \mu}{\partial t} = -\frac{(\mu_{\text{marker}} - \mu_A) (t_{\text{marker}} - \lambda t_R) (t_A - \lambda t_R)}{t_A - t_{\text{marker}}} \cdot \frac{1}{(t - \lambda t_R)^2}. \tag{28}
\]

Mobilograms should take into account this factor, giving a corrected mobilogram intensity \( I_{\text{mob}}^i \),

\[
I_{\text{mob}}^i = (t_i - \lambda t_R)^2 I_i \times \frac{t_A - t_{\text{marker}}}{(\mu_{\text{marker}} - \mu_A) (t_{\text{marker}} - \lambda t_R) (t_A - \lambda t_R)}. \tag{29}
\]

The areas obtained by integrating the electropherogram \((t_i, I_i)\) and the corrected mobilogram \((\mu(t_i), I_{\text{mob}}^i)\) will therefore be the same.

The right factors in (29) do not depend on the specific point of the mobilogram, and it is a function of only \( L, E_m, t_R \) and \( v_p \). This means that when comparing runs performed under the same instrumental conditions (field magnitude and ramp, capillary length, and pressure), we can do without such overall factor, simply using

\[
I_{\text{mob}}^i \propto (t_i - \lambda t_R)^2 I_i. \tag{30}
\]

The previous derivation has been devoted to ensuring that areas in mobilograms map faithfully to areas in electropherograms. Without further treatment, this assumes that in turn electropherogram areas represent faithfully the amount of substance in the sample.
This is highly dependent on the detection type. In the context of CE-MS, the critical element is the electrospray ionization (ESI) that nebulizes the output of the capillary to be fed into the MS. This ionization has two well-known regimes of operation, the so-called mass and concentration modes [13]. In mass mode, the ESI is able to ionize (nearly) all the substance coming from the capillary. If the capillary flow is increased, more substance will be ionized per unit time and the MS will simply register more counts. By contrast, when in concentration mode the ESI is saturated. It outputs an amount of ions proportional to the volumetric concentration of substance arriving from the capillary, independently of time. Crucially, increasing the flow in the capillary does not increase the number of counts.

The increase in width between the two flow regimes is strictly proportional to the exit speed of the analyte from the capillary – this does not depend on the ionization mode, simply on the fact that a fixed length in the capillary spends twice as much time exiting it if it is moving at half the speed. One can emulate the height reduction observed in mass mode on data measured in concentration mode by simply multiplying each peak by its speed. Equation (17) relates the speed of an analyte to its mobility, which in turn can be derived from its migration time from (23) or (24). This provides the exit speed \( v_{exit} \) as a function of migration time \( t_M \),

\[
v_{exit}(t_M) = \left( L - \lambda t_R v_p \right) \cdot \frac{1}{t_M - \lambda t_R}
= \frac{E_m (\mu_{marker} - \mu_A)(t_{marker} - \lambda t_R)(t_A - \lambda t_R)}{t_A - t_{marker}} \cdot \frac{1}{t_M - \lambda t_R}. \tag{31}
\]

As a side note, detection from UV absorbance and similar methods work exactly in the same way concentration mode ESI does in CE-MS. The absorbance is not a function of the flow rate, so total peak areas will depend on it. The same correction applies to them.

So, if one has an electropherogram measured in concentration mode (or from UV measurements), given by couples \((t_i, I_{i,conc}^\text{conc})\), the equivalent mass-mode electropherogram can be computed with

\[
I_{i,\text{mass eq.}} = I_{i,\text{conc.}} \cdot v_{exit}(t_i) = \frac{I_{i,\text{conc.}}}{t_i - \lambda t_R} \times \frac{E_m (\mu_{marker} - \mu_A)(t_{marker} - \lambda t_R)(t_A - \lambda t_R)}{t_A - t_{marker}}. \tag{32}
\]

Just like for the mobility transformation correction (9), when comparing runs under the same experimental conditions one may as well ignore the constant factor and use

\[
I_{i,\text{mass eq.}} \propto \frac{I_{i,\text{conc.}}}{t_i - \lambda t_R}. \tag{33}
\]

### E.3 Experimental validation of the proposed formulas

To follow each compound along the different pressure, the replicates were averaged out,

\[
t_{c,p}^{(\text{average})} = \frac{\sum_{i} t_{c,p,i}}{N_{\text{replicates}}}. \tag{34}
\]

and in order to compare the compounds against each other, normalized by their own mean over all pressures,

\[
t_{c,p}^{(\text{normalized})} = \frac{t_{c,p}^{(\text{average})}}{\sum_{p'} t_{c,p'}^{(\text{average})}/N_{\text{pressures}}}. \tag{35}
\]
The same transformations were applied to obtain an array of normalized areas per compound and pressure, $A_{c,p}^{\text{normalized}}$. These normalized values track only the variation between the free parameter (in this case, the pressure) relative to the compound’s overall mean, so that if for some compound $t_{c,p}^{\text{average}}$ would not change at all between pressures, $t_{c,p}^{\text{normalized}} = 1$ for all $p$.

To ensure that areas respond linearly to the amount of substance in the mix, it was analyzed from preparations at four concentration levels (62.5, 125, 250, and 500 ppb), each under three pressures (10, 30, and 50 mbar), which in turn were also run in triplicate. As by now we know that different pressures should provide the same areas, we average both over replicates and pressures,

$$A_{c,\rho}^{\text{average}} = \sum_{p} \sum_{i} A_{c,\rho,p,i} / \left( N_{\text{replicates}} \cdot N_{\text{pressures}} \right),$$

and normalize over the free variable, the concentration $\rho$,

$$A_{c,\rho}^{\text{normalized}} = \frac{A_{c,\rho}^{\text{average}}}{\sum_{\rho'} A_{c,\rho'}^{\text{average}} / N_{\text{concentrations}}}. \quad (37)$$