Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

Received: 13 October 2021
Revised: 22 November 2021
Accepted: 23 November 2021

Profiling acidic metabolites by capillary electrophoresis-mass spectrometry in low numbers of mammalian cells using a novel chemical derivatization approach

Marlien van Mever[1,*] | Cornelius C. W. Willacey[1,*] | Wei Zhang[1] | Nicolas Drouin[1] | Alphert E. Christina[2] | Peter W. Lindenburg[1,2] | Jaco P. D. van Veldhoven[1] | Daan van der Es[1] | Amy C. Harms[1] | Thomas Hankemeier[1,*] | Rawi Ramautar[1,*]

[1] Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

[2] Research Group Metabolomics, Leiden Centre for Applied Bioscience, University of Applied Sciences Leiden, Leiden, The Netherlands

Correspondence
Dr. R. Ramautar, Biomedical Microscale Analytics, Leiden Academic Centre for Drug Research (LACDR), Leiden University, 2333 CC Leiden, The Netherlands.
Email: r.ramautar@lacdr.leidenuniv.nl

[*] M. van Mever and C. C.W. Willacey are equally contributed first authors, and T. Hankemeier and R. Ramautar are contributed equally as last/senior authors.

Funding information
Netherlands Organization of Scientific Research, Grant/Award Number: NWO Vidi 723.016.003; Horizon 2020 Marie Sklodowska-Curie, Grant/Award Number: 707404

Abstract

The simultaneous analysis of a broad range of polar ionogenic metabolites using capillary electrophoresis-mass spectrometry (CE-MS) can be challenging, as two different analytical methods are often required, that is, one for cations and one for anions. Even though CE-MS has shown to be an effective method for cationic metabolite profiling, the analysis of small anionic metabolites often results in relatively low sensitivity and poor repeatability. In this work, a novel derivatization strategy based on trimethylmethaneaminophenacetyl bromide was developed to enable CE-MS analysis of carboxylic acid metabolites using normal CE polarity (i.e., cathode in the outlet) and detection by mass spectrometry in positive ionization mode. Optimization of derivatization conditions was performed using a response surface methodology after which the optimized method (incubation time 50 min, temperature 90°C, and pH 10) was used for the analysis of carboxylic acid metabolites in extracts from HepG2 cells. For selected metabolites, detection limits were down to 8.2 nM, and intraday relative standard deviation values for replicates (n = 3) for peak areas were below 21.5%. Metabolites related to glycolysis, tricarboxylic acid cycle, and anaerobic respiration pathways were quantified in 250,000 cell lysates, and could still be detected in extracts from only 25,000 HepG2 cell lysates (~70 cell lysates injected).

KEYWORDS
capillary electrophoresis, carboxylic acid metabolites, chemical derivatization, HepG2 cells, metabolomics

Abbreviations: AKG, α-ketoglutaric acid; CCD, central composite design; CITS, citric acids; DmPABr, dimethylaminophenacyl bromide; FUM, fumaric acid; GA, glutaric acid; ICD, isotope-coded derivatization; IM, ion mobility; LAC, lactic acid; LOF, lack-of-fit; MAL, malic acid; ME, matrix effect; MEF, matrif effect factor; MNA, malonic acid; MT, migration time; OXA, oxaloacetic acid; PIESI, paired ion electrospray ionization; PYR, pyruvic acid; RSM, response surface methodology; SIL, stable-isotope labeled; SL, sheath-liquid; SUC, succinic acid; TCA, tricarboxylic acid; TEOA, triethanolamine; TmAmPBr, 4-(trimethylammoniomethyl)phenacyl bromide

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

1 | INTRODUCTION

The metabolome consists of a vast number of components, including a wide variety of compound classes, such as amino acids, organic acids, nucleotides, fatty acids, and so on. Profiling of small (endogenous) molecules in biological matrices, coined as metabolomics, is of fundamental importance to elucidate the cellular metabolism under pathophysiological conditions. The diversity in chemical and physical properties of the metabolites, as well as the broad concentration range in which they are present in different biological matrices, makes their (simultaneous) analyses challenging. Analytical techniques that are often used for metabolomics studies include nuclear magnetic resonance (NMR) spectroscopy,[1] and liquid chromatography (LC)[2] or gas chromatography (GC)[3] coupled to mass spectrometry (MS).[4] In recent years, capillary electrophoresis coupled to mass spectrometry (CE-MS) gained attraction in the scientific community for metabolomics, notably for the analysis of polar ionogenic metabolites given its separation mechanism, but also for the analysis of volume-limited biological samples,[5–9]

Within the past decade, CE-MS has been highlighted as an effective method for cationic metabolite profiling, thereby applying normal CE polarity and detection by MS in positive ionization mode,[10] as exemplified by a recent study that included a large cohort of over 8000 biological samples,[10] and also by the recent Metabo-ring trial,[11] which both revealed that CE-MS is a viable and reproducible approach for metabolomics. On the other hand, the profiling of anionic metabolites by CE-MS has been considered a challenging endeavor.[12] Anionic metabolic profiling is usually performed by a method first proposed by Soga et al,[13] where a cationic polymer-coated capillary in combination with a weakly alkaline ammonia buffer is used, thereby employing reversed CE polarity and negative ionization mode. However, the long-term stability of the cationic coated capillary proved to be relatively poor,[14] which was mainly due to corrosion of the stainless steel ESI needle when employing reversed CE polarity and negative ionization mode conditions. Additionally, a cross-platform study revealed that the use of a reverse CE polarity in conjunction with MS detection in negative ionization mode for anionic metabolite profiling resulted in relatively poor metabolite responses and repeatability.[15] By replacing stainless steel for platinum as ESI spray needle greatly improved method performance and long-term stability when employing CE with cationic-coated coatings under reversed polarity for anionic metabolic profiling.[14] However, a platinum needle is not required for anionic metabolic profiling under normal CE polarity conditions as electrolytic corrosion at the anode is avoided. Recently, Yamamoto et al showed that commonly used ammonium acetate or ammonium formate background electrolytes (BGEs) with a pH above 9.0 contributed to incidental capillary fractures via irreversible aminolysis of the outer polyimide coating.[16] Prevention of polyimide aminolysis could be easily achieved by using weakly alkaline, ammonia-containing buffers (pH < 9.0).

To circumvent the issues described above, new efforts have been made to increase the performance of CE-MS for anionic metabolite profiling. For example, Lee *et al*. proposed a novel strategy based on the use of ion-pair reagents for analysis of short-chain fatty acids in the positive ion mode by MS.[17] The so-called paired ion electrospray ionization (PIESI)-MS allowed for lower detection limits and higher repeatability compared to analysis in negative ionization mode. However, ion suppression could still occur due to the ion-pair reagents. Zhang et al developed a sheathless CE-MS method for the profiling of nucleotides in the positive ion mode and employing a BGE of ammonium acetate with pH 9.7 with electrophoretic separation in normal polarity mode.[18] Another strategy was recently proposed by Drouin et al, utilizing a two-step CE-MS method for the analysis of cations and anions by using the same analytical conditions,[19] except only reversing the CE polarity. Some anions were detected as their ammonium adducts, and higher sensitivity was achieved than when using MS detection in negative ionization mode. Though the same MS conditions could be used, a CE polarity switch was still required.

Chemical derivatization is a sample preparation technique that subjects target compounds to chemical reactions with a derivatization reagent and provides them more favorable physicochemical properties,[20] such as changes in polarity,[21,22] separation, stability, ionization, and vulnerability to ion suppression. Chemical derivatization is most commonly applied to GC-MS and LC-MS.[21,23–26] Surprisingly, there is a great minority in CE-MS applications, while the use of chemical derivatization could enhance ionization.[21,22,24,25,27] Various efforts to analyze anionic metabolites as cationic analytes by CE-MS using chemical derivatization have been developed over the past years. For example, Yang et al developed a relatively time-consuming (2 h) two-step derivatization approach for the analysis of carboxylic acid-containing metabolites in urine using *N*-butyl-4-aminomethylpyridinum iodide and *N*-hexyl-4-aminomethyl-pyridinum iodide.[28] D'Agostino et al used the reagent maleimide to label thiols in plasma. However, the derivatization using maleimide was restricted to the analysis of thiols only.[29] More recently, Huang et al proposed a strategy for the derivatization of amines, hydroxyls, and carboxylates using a two-stage derivatization using 3-(Diethylamino) propionyl chloride and *N*-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b] pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide.[30]

In this study, a novel derivatization strategy based on trimethylmethaneaminophenacetyl bromide (TmAmPBr) was developed to enable CE-MS analysis of carboxylic acid-containing metabolites using normal CE polarity and MS detection in positive ionization mode. The use of TmAmPBr, which contains a quaternary amine group, provides derivatives with a permanent positive charge, which is beneficial in terms of ionization efficiency. The mechanism of action of this reagent is similar to the methods published by Willacey et al[21,25] and Guo et al[31]; in which dimethylaminophenacyl bromide (DmPABr) was used for the labeling of carboxylic acids. Though the reaction mechanism follows the same S_n2 reaction kinetics as phenacyl bromide and DmPABr (Figure 1), further optimization of the derivatization conditions using TmAmPBr has been carried out to ensure that reliable derivatization could be achieved in the presence of the quaternary amine using a response surface methodology (RSM). As a first step, the derivatization for metabolites containing a carboxylic acid group was investigated,

Research Article
doi.org/10.1002/ansa.202100054

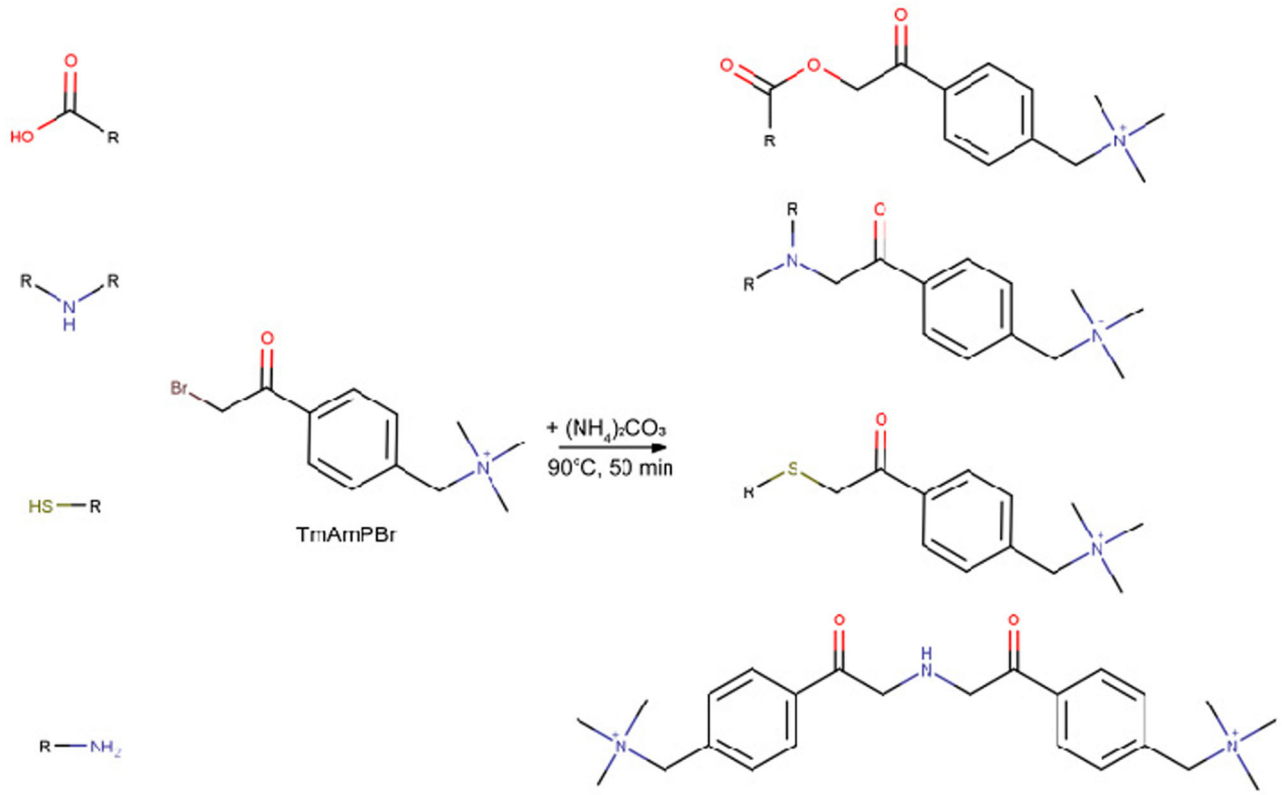

FIGURE 1 S_N2 reaction scheme of TmAmPBr with the carboxylic acid, thiol, secondary amine, and primary amine, respectively

since these metabolites are currently the most challenging to analyze using CE-MS. For method development, 11 acidic metabolites associated with glycolysis, tricarboxylic acid (TCA) cycle, and anaerobic respiration were selected and the analytical performance was assessed by considering aspects like linearity, repeatability, limit of detection (LOD), and limit of quantification (LOQ). The applicability of the overall method was demonstrated for the analysis of acidic metabolites in extracts from low numbers of HepG2 cells.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

All used chemicals were of analytical grade or higher. Paracetamol and oxaloacetic acid were acquired from Fluka (Buchs, Switzerland). Ammonium carbonate was purchased from Merck (Darmstadt, Germany). Glutaric acid, procaine, malonic acid, pyruvic acid, α-ketoglutaric acid, fumaric acid, isocitric acid, succinic acid, malic acid, citric acid, lactic acid, triethanolamine (TEOA), and dimethyl sulfoxide (DMSO) were from Sigma–Aldrich (Steinheim, Germany). Deuterated stable-isotope labeled (SIL) standard succinic acid-d6 was from Cambridge Isotope Laboratories (Apeldoorn, the Netherlands). Hydrochloric acid (37% m/m) was acquired from Thermo Fisher Scientific (Waltham, MA, USA). Acetic acid and propan-2-ol were from Biosolve (Valkenswaard, the Netherlands). Acetonitrile (MeCN) and dimethylformamide (DMF) were purchased from Actu-All Chemicals (Oss, The Netherlands) and Honeywell (Rosmalen, The Netherlands), respectively. The procedure for the synthesis of TmAmPBr is described in Supporting Information S1.

A Milli-Q Advantage A10 water purification system (Merck, Darmstadt, Germany) was used to obtain pure water. Metabolite standard solutions (1 mg/mL) were prepared in DMF/DMSO (50/50 v/v). Working standard mixtures of 12 compounds (500 µM) were prepared by dilution with DMF/DMSO, aliquoted, and stored at -80°C. Internal standard succinic acid-d6 was prepared in DMF/DMSO (125 µM), and internal standards paracetamol and procaine were prepared in BGE (50 µM). Internal standards were aliquoted and stored at -80°C, and internal standard stock aliquots were thawed on the day of use. Ammonium carbonate was prepared in water to the desired concentrations. When necessary, the pH was adjusted using a 1 M sodium hydroxide solution. As BGE solution, 10% (v/v) acetic acid (1.7 M) in water was used. Sheath-liquid consists of water and propan-2-ol (50:50, v/v), supplemented with 0.03% (v/v) acetic acid.

2.2 | Derivatization optimization by RSM

The derivatization procedure was optimized using an RSM. As a first step, a suitable alkaline solution was selected based on compatibility

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

with CE-MS analysis, thereby comparing triethanolamine and ammonium carbonate. Subsequently, a screening design was used to determine the main variables affecting the derivatization procedure. A two-level (2^{5-1}) fractional factorial design was built with five variables ammonium carbonate pH (X1), ammonium carbonate concentration (X2), incubation temperature (X3), incubation time (X4), and shaking speed (X5), resulting in an experimental design of 16 trials at low (-1) and high (+1) levels (Supporting Information S2). The screening design was used to evaluate the relative magnitude of the statistical significance of the effects by Pareto charts. Thereafter, a Central Composite Design (CCD) was built for optimization of the derivatization procedure. Each factor was set to five levels: plus and minus α (axial points), plus and minus 1 (factorial points), and the center point, resulting in a total of 50 experiments, that is, 42 design experiments with eight replicate runs at the center point (Supporting Information S3). The experiments were carried out in a random sequence to minimize bias and to reduce the outcomes of unpredicted variability in the responses. The responses were peak area ratios (corrected for the internal standard) of derivatized fumaric acid (Y1), oxaloacetic acid (Y2), α-ketoglutaric acid (Y3), and pyruvic acid (Y4). The statistical and graphical interpretation of the effects was performed by using the Design Expert Software (version 12, Stat Ease Inc., MN, USA). Optimal values of the variables for derivatization were obtained using Derringer's desirability (D).

2.3 | Mammalian cell lysate sample collection and preparation

Human liver cancer cells (HepG2) cells were cultured, harvested, and collected in-house as described previously in ref. 18. First, the harvested cells were counted using a TC10 Automated Cell Counter (Bio-Rad Laboratories), and the live cell density was at 7.4×10^6 cells/mL. Pre-heated (37°C) culture medium (5 mL) was added to the Petri dishes (60 mm, n = 3), and after the dispersion of the cell mixture, 135 µL of the cell mixture (~10^6 live cells) was added to the medium. The Petri dishes were gently shaken to distribute the cells evenly before incubation (37°C in 95% air/5% CO_2). After the cells adhered to the bottom of the Petri dishes (after ~7.5 h), the dishes were taken out of the incubator, the medium was aspirated and pre-heated (37°C) PBS (6 mL) was used to wash away residual culture medium, after which the PBS was removed. To quench intracellular enzymatic reactions, ice-cold methanol/H_2O (80:20, v/v) mixture (1 mL) was added into every Petri dish. The dishes were placed on ice, and scraped in order to get all the cells off the surface. The HepG2 cells were collected in aliquots of 2×10^6 cells per vial in Eppendorf vials and stored at -80°C until sample preparation was performed. The cell supernatant was aliquoted into new Eppendorf vials equivalent to 5×10^5, 2.5×10^5, 1×10^5, 5×10^4, 2.5×10^4, 1×10^4, and 5×10^3 cells. Sample clean-up to remove lipid and protein contents was performed by a liquid-liquid extraction procedure using ice-cold water/methanol/chloroform (1/1/1, v/v/v). The methanol/water phase was collected, evaporated, and then reconstituted in 10 µL DMF/DMSO that was used for derivatization.

2.4 | Derivatization and quantification of mammalian cells for sensitivity analysis

The described derivatization parameters were optimized using RSM (Section 2.2). HepG2 cell supernatants were dried using Labconco SpeedVac (MO, United States). Dried residues were reconstituted in 10 µL DMF/DMSO (50/50 v/v). Then subsequent additions were made of 10 µL ammonium carbonate (65 mM, pH 10), 5 µL internal standard solution (125 µM) and 20 µL TmAmPBr (40 mg/mL), with vortexing after each addition. Subsequently, the Eppendorf vial was placed into a shaking incubator at maximum speed (900 RPM) for 50 min at 90°C to ensure thorough derivatization. After incubation, 10 µL acetic acid (10% v/v, 1.7 M) was added to the vial to quench the reaction, followed by an additional 20 min of incubation under the same conditions.

2.5 | CE-MS analysis

CE-MS analyses were performed on a 7100-capillary electrophoresis system from Agilent Technologies (Waldbronn, Germany), hyphenated with an Agilent 6230 TOF (Santa Clara, CA, USA). A co-axial sheath-liquid ESI interface coupled via a triple-tube sprayer was used. Sheath-liquid was delivered at a flow rate of 3 µL/min by an Agilent 1260 Infinity Isocratic Pump (Agilent Technologies) with a flow splitter (ratio 1:100). Fused silica capillaries (internal diameter: 50 µm) were purchased from BGB Analytik (Harderwijk, the Netherlands), and cut manually to a length of 70 cm. Conditioning of new capillaries was done by subsequently rinsing with methanol, water, sodium hydroxide 1 M, water, hydrochloric acid 1 M, water, hydrochloric acid 0.1 M, water, and BGE, each at 5 bar for 1 min. Injections were performed hydrodynamically for 24 s at 50 mbar (27.4 nL), and volumes were calculated with Zeecalc v1.0b (https://epgl.unige.ch/labs/fanal/zeecalc). The separation voltage was 30 kV and additional pressure was applied (40 mbar) during separation.

CE-MS experiments were acquired in positive ionization mode, between 65 and 1000 *m/z* with an acquisition rate of 1.5 spectra/s. The following MS settings were used; nebulizer gas: 0 psi, sheath gas (nitrogen) flow rate: 11 L/min, sheath gas temperature: 100°C, ESI capillary voltage: 5500 V, fragmentor voltage: 150 V, skimmer voltage: 50 V, as adapted from previous work.[8,32] From the sheath-liquid, propan-2-ol $[C_3H_8O+H]^+$ and its clusters $[(C_3H_8O)_2+H]^+$ and $[(C_3H_8O)_3+H]^+$ with corresponding *m/z* values of 61.06479, 121.12231, and 181.17982, respectively, were used as lock masses. For data acquisition and treatment, MassHunter version B.06.00 (Agilent, Santa Clara, CA, USA) was used. Peak extraction was performed with a mass error of 20 ppm and peak integrations were visually inspected to ensure correct integration.

2.6 | Analytical performance evaluation

The optimized derivatization procedure was evaluated in terms of calibration curves, repeatability, limits of quantification (LOQ), and limit

of detection (LOD) as well as matrix effect. The response function of the measured peak area for 11 carboxylic acid metabolites (See Supporting Information S4) was examined using three replicates at eight different concentration points over a concentration range from 0.7 to 90 µM. As an internal standard, SIL succinic acid-d6 (25 µM) was used. Intraday repeatability (expressed as percentage relative standard deviation, % RSD) was evaluated for replicates on the same day (n = 3). The LOD and LOQ were estimated as 3 σ/slope and 10 σ/slope, respectively (σ = standard deviation of the lowest calibration point).

To evaluate matrix effect regarding TmAmPBr derivatization and CE-MS analysis, the matrix effect factor (MEF) was determined for SIL succinic acid-d6, which was spiked into the sample after extraction, but before derivatization. MEF was calculated as follows:

$$\text{Matrix effect factor (MEF)} = \frac{\overline{AUC_{\text{water}}} - AUC_{\text{cells}}}{\overline{AUC_{\text{water}}}} * 100$$

where $\overline{AUC_{\text{water}}}$ is the averaged area of the SIL-internal standard in standards and AUC_{cells} is the peak area of the SIL-internal standard in a cell sample. MEF was calculated for all calibration points.

3 | RESULTS & DISCUSSION

3.1 | Optimization of reaction conditions

3.1.1 | Initial screening of reaction parameters

Initially, the optimized reaction conditions for derivatization with DmPABr were adapted for TmAmPBr derivatization (incubation time: 60 min, incubation temperature: 65°C).[25] Four carboxylic acid metabolites with different physicochemical properties, namely, pyruvic acid, oxaloacetic acid, α-ketoglutaric acid, and fumaric acid (Supporting Information S4), were selected as test analytes for optimization of the reaction conditions. Two types of derivatives, singly derivatized and doubly derivatized acids (Supporting Information S5), were observed for oxaloacetic acid, α-ketoglutaric acid, and fumaric acid, since there are two carboxylic acid groups present on these compounds, whereas for pyruvic acid only the single derivatized acid was detected, as pyruvic acid only contains one carboxylic acid group. Since the doubly derivatized acids contain two positive charges, they migrated faster and appeared at the front in the electropherogram, followed by singly derivatized acids. Probably, singly derivatized acids are still observed for oxaloacetic acid, α-ketoglutaric acid, and fumaric acid, due to incomplete derivatization, or because of sterical hindrance during the derivatization reaction. Additionally, peak intensity and shapes were more desirable for doubly derivatized metabolites, and therefore, for oxaloacetic acid, α-ketoglutaric acid, and fumaric acid, the doubly derivatized forms were selected for further optimization experiments, whereas for pyruvic acid, the singly derivatized variant was evaluated. The presence of singly derivatized and doubly derivatized acids could be detrimental for the detection sensitivity of diprotic carboxylic acids, as the signal for a certain analyte is splitted. This issue was further investigated under optimized conditions. Preliminary analysis showed that derivatization was achieved adapting the protocol that was developed for derivatization with DmPABr, however, with undesirable peak shapes and signal intensities, and a large distortion in the total ion electropherogram TIE signal was observed. A closer inspection of the recorded mass spectra revealed ion suppression presumably attributed to the use of TEOA (*m/z* 150.112) as a base catalyst. Additionally, the high conductivity of TEOA could cause a destacking effect during CE-MS analysis, thereby causing poor peak shapes. Therefore, ammonium carbonate was proposed as base catalyst due to its compatibility with ESI-MS, relatively high pH buffering range, and lower conductivity. More favorable peak shapes and an increase of signal intensity were observed for the selected compounds when ammonium carbonate (100 mM, pH 9.2) was used, as shown by an increase in peak area by 89-99% (Supporting Information S6). When using the water-soluble base ammonium carbonate as the base catalyst for the reaction, the total elimination of water content is no longer realized. However, previous research has shown that a water content of <40% does not significantly change the derivatization efficiency for derivatization with DmPABr,[21] and is also not essential for the derivatization of carboxylic acids. Due to the similarity of DmPABr and TmAmPBr, it was expected that the water content (~22%) does not significantly impact the reaction efficiency, as confirmed experimentally (Supporting Information S7). Pyruvic acid is shown to be more impacted by the presence of water when compared to the other organic acids. This may be due to the higher reaction energy required between ketoacids and TmAmPBr. Other than this, the potential small decrease in reaction efficiency is a reasonable compromise for the large reduction of ion suppression.

Thereafter, a multivariate screening design (two-level fractional factorial design, see Supporting Information S2 for factors and factor levels) was performed to determine significant parameters for the derivatization procedure. Earlier studies that evaluated derivatization strategies revealed that various experimental parameters have an effect on the efficiency of derivatization, such as pH of the reaction solution, reaction duration, and reaction temperature.[21,30,33–35] Therefore, these parameters were selected as factors for the screening design. Additionally, the stirring speed during the reaction was added as a factor.[36] As previously mentioned, the water content in the reaction mixture was kept constant (~22%). The amount of derivatization reagent was also kept constant at 40 mg/mL to ensure an excess in reagent and thus complete derivatization.[37] The screening design experiments were performed, and the main effects of the five studied variables are presented in the form of Pareto charts (Supporting Information S8). The magnitude of the effects is displayed on the bar charts, and the vertical axis shows the accompanying t-values. The plots indicate that all five investigated factors are above the *t*-value limit, and are therefore possibly important. Therefore, for the optimization experiments, all factors were considered. Factor levels, as listed in Supporting Information S3, were selected in accordance with previous studies.[21]

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

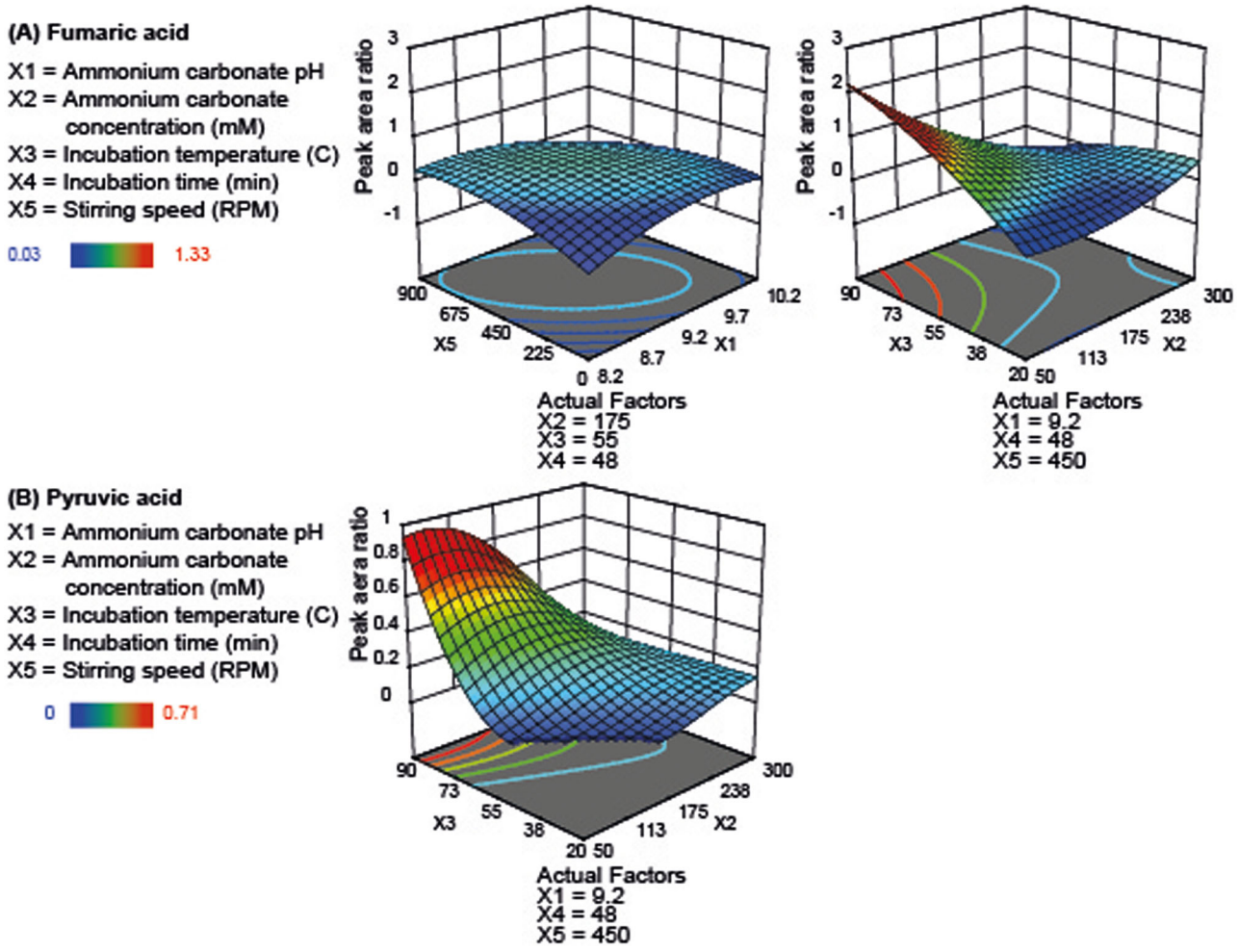

FIGURE 2 Optimization design 3D surface plots for (A) fumaric acid and (B) pyruvic acid

3.1.2 | Optimization of reaction conditions using RSM

For further optimization of the reaction conditions for derivatization of carboxylic acid metabolites using TmAmPBr. CCD experiments were conducted. Regression analysis was performed to fit the response function, and non-significant terms were eliminated to simplify the models, leading to reduced models for all responses (Supporting Information S9). ANOVA results showed that factor X3 (incubation temperature) and interaction factor between factors X2 and X3 (ammonium carbonate concentration X incubation temperature) were significant for all responses ($p < 0.05$) and thus can be considered as the most important factors for the derivatization process. According to the analysis of variance (ANOVA) results and 3D response surface plots (Figure 2), two-factor interaction effects were assumed. Therefore, a multi-factor optimization is desirable for the optimization of this derivatization procedure. Factor X5 (stirring speed) showed no significant effect for the responses, so was fixed at maximum speed (900 RPM). According to 3D response surface plots (Figure 2), a high incubation temperature (>80°C) and incubation time of at least 40 min are necessary for the formation of derivatized acids. To determine the optimal parameters for the derivatization process, Derringer's desirability multi-criteria decision making was applied.[38] The goal of this optimization was to find a set of conditions with the best compromise between the individual goals, that is, the highest peak area for all four tested metabolites. Desirability plots combining these goals are shown in Supporting Information S10, where a higher D-value denotes a more favorable option. The optimal response was achieved with ammonium carbonate pH > 9.4, ammonium carbonate concentration between 50 and 100 mM, incubation temperature > 80°C, incubation time > 40 min, and stirring speed of 900 RPM. Five optimal parameter settings were tested in order to verify the predicted responses and determine the optimal method (option 1: ammonium carbonate pH 10, ammonium carbonate concentration of 65 mM, incubation temperature 90°C, incubation time of 50 min, and stirring speed of 900 RPM, option 2: ammonium carbonate pH 9.8, ammonium carbonate concentration of 85 mM, incubation temperature 90°C, incubation time of 75 min, and stirring speed of 900 RPM, option 3: ammonium carbonate pH 9.6, ammonium carbonate concentration of 70 mM, incubation temperature 80°C, incubation time of 40 min, and stirring speed of 900 RPM, option 4: ammonium carbonate pH 9.4, ammonium carbonate concentration of 55 mM, incubation temperature 90°C, incubation time of

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

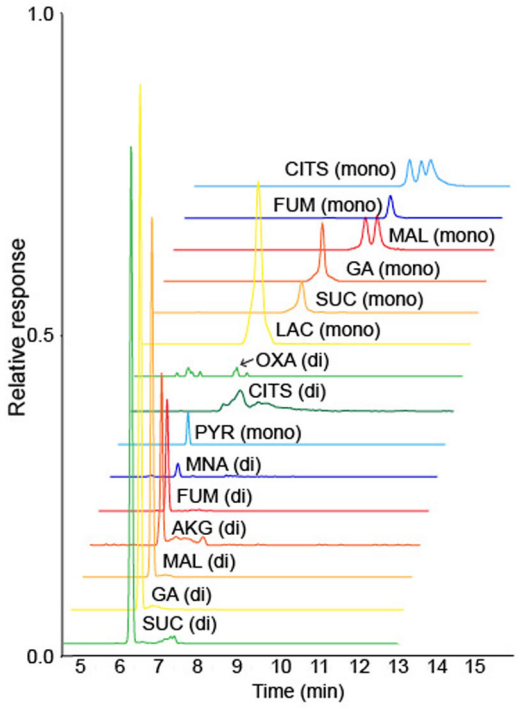

FIGURE 3 Extracted-ion electropherogram obtained by CE-MS from the analysis of 12 metabolites (90 µM) after derivatization with TmAmPBr. Separation conditions: BGE, 10% (v/v) acetic acid (1.7 M); sample injection volume 27 nL

45 min, and stirring speed of 900 RPM, option 5: ammonium carbonate pH 9.2, ammonium carbonate concentration of 60 mM, incubation temperature 90°C, incubation time of 40 min, and stirring speed of 900 RPM) for organic acid test mixtures (500 µM) composed of 11 organic acids (Supporting Information S4 and S5) with different physicochemical properties. The highest signal intensities for target metabolites were obtained with option 1: ammonium carbonate pH 10, ammonium carbonate concentration of 65 mM, incubation temperature 90°C, incubation time of 50 min, and stirring speed of 900 RPM. For this reason, these conditions were used for follow-up studies.

3.2 | Analytical performance evaluation

3.2.1 | Method performance in standard solutions

After determining the optimal conditions for the derivatization of carboxylic acid metabolites with TmAmPBr, the analytical performance of the method (including the derivatization procedure and CE-MS method) was evaluated by preparing dilution series, LODs, LOQs, and peak area repeatability for a mixture of test compounds.

Figure 3 shows extracted ion electropherograms obtained for the analysis of derivatized metabolites by CE-MS. As already stated above, two types of derivatives, singly derivatized (mono) and doubly derivatized (di) acids (see Supporting Information S5), were observed for most organic acids including fumaric acid, succinic acid, malic acid, and glutaric acid, due to the presence of two carboxylic acid groups. On the other hand, for pyruvic acid and lactic acid only the single derivatized acid was detected, as these compounds only contain one carboxylic acid group. For malonic acid, oxaloacetic acid, and α-ketoglutaric acid, the mono-form was not detected under the current conditions, or showed a very low response (< 500 counts), and were therefore excluded from Figure 3. For citric acid and isocitric acid, only the single and double derivatized species were detected, whereas theoretically, three labels could be added due to three carboxylic acid groups. The triple derivatized species is not formed, which is possibly due to steric hinderance. In addition, a repulsion effect may occur due to the high density of positive charge caused by the addition of the quaternary amines on TmAmPBr. Interestingly, for compounds malic acid and citric/isocitric acid, multiple peaks were observed for the singly derivatized species. This is possibly due to the asymmetry of the molecules, therefore showing different labeled species. This could be further examined by for instance using MS/MS or ion mobility MS. For the acids which show both single and double derivatized species, the ratio between the mono- and di-form was investigated, and showed to be stable across the linear range 0.7-90 µM (at concentrations < 5.7 µM, the mono-form was not detected), e.g 1:3 for fumaric acid and 1:4 for glutaric acid 1:5 for malic acid and 1:4 for succinic acid, for singly:doubly derivatized species, respectively.

For low molecular mass compounds, the CE separation is driven by the difference in ionization in solution. Since the derivatization blocks the ionizable function and brings a permanent charge, the separation is only mediated by the difference in hydrodynamic radius. This explains why all compounds with the same degree of derivatization are migrating in a very narrow time range, for example, fumaric acid (migration time (MT) 6.64 min), succinic acid (MT 6.63 min), and malic acid (MT 6.65 min). As TOF-MS was used for detection, it was still possible to make a selective distinction between the closely migrating metabolites. Citric acid and isocitric acid were not completely baseline resolved (resolution = 1.1). However, in this work, the emphasis was on the optimization of the derivatization procedure and on its value for the profiling of acidic metabolites in positive ion mode by CE-MS.

The stability of the derivatives was examined by analyzing derivatized calibration standards (90 µM) that were stored for 24 h in the autosampler tray (22°C), and also for one month at -18°C and at -80°C. The samples were then compared against freshly derivatized samples. No difference was observed for peak areas (Student's *t*-test at 95% CI, paired), as expected according to previous research.[31]

Eight-point dilution series, including replicates in order to include variations in sample preparation, was established for standards, and SIL succinic acid-d_6 (25 µM) was used as internal standard. A linear response (and with $R^2 > 0.961$) for model compounds was obtained in the concentration range from 0.7 to 90 µM, and linearity was further evaluated by a lack-of-fit (LOF) test,[39] and the method yielded linearity for all test compounds. Repeatability of the derivatization procedure for standards was assessed for replicates based on the CE-MS analyses of standards at two concentration levels (highest and lowest calibration points) (Table 1). The repeatability of the derivatization was determined by performing the derivatization procedure in parallel (n = 3).

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

TABLE 1 Summary of linear range, the *F*-value of the Lack-of-fit (LOF) test (Fcrit, 95% ≥ 2.74) and limit of detection (LOD), of 8-point calibration lines for metabolite standards. Repeatability was determined for the highest and lowest calibration points. See Section 2 for experimental conditions

Compound	Range (μM)	LOF	LOD (nM)	Repeatability at the highest concentration (n = 3) (% RSD)	Repeatability at the lowest concentration (n = 3) (%RSD)
Pyruvic acid (mono)	0.7-90	0.9	28.7	10.0	7.4
Fumaric acid (di)	0.7-90	1.4	8.2	13.8	5.8
Glutaric acid (di)	0.7-90	0.6	16.4	10.9	14.0
Citric acids (di)*	11.4-90	1.6	52.3	10.7	34.8
Malic acid (di)	0.7-90	1.8	8.8	12.2	10.5
Succinic acid (di)	0.7-90	0.5	89.5	9.1	4.8
Lactic acid (mono)	0.7-90	1.8	72.9	7.1	3.1
Oxaloacetic acid (di)	5.7-90	0.03	610	13.1	16.5
Malonic acid (di)	1.4-90	0.4	56.9	17.0	1.2
α-Ketoglutaric acid (di)	1.4-90	0.1	49.7	21.5	6.4

*Isocitric and citric acid (di) were not baseline separated and integrated as one peak.

Intraday RSD values (n = 3) for internal standard corrected peak areas of all analytes were better than 21.5% for the highest calibration point, and below 16.5% for the lowest calibration point, except for the citric acids (36.2%), that were integrated as one peak. Considering this is the repeatability for both the experimental and analytical variation, the obtained numbers are acceptable.

Detection limits were calculated estimated on the dilution series as 3 σ/slope and 10 σ/slope, respectively (σ = standard deviation of the lowest calibration point), and were ranging from 8.2 to 610 nM for carboxylic acid standards (Table 1). Compared to other CE-MS studies detecting carboxylic acids,[13,14] we observed an improvement of 10-100 times in estimated detection limits by using TmAmPBr derivatization. This improvement is probably due to the switch in MS ionization polarity from negative to positive mode, thereby allowing for the use of low pH BGE, bare-fused silica capillaries, and a stainless steel ESI needle, creating stable electrospray.

3.2.2 | Applicability to mammalian cells

The applicability of the optimized method for the analysis of acidic metabolites in biological samples was evaluated using HepG2 cells as a model system. For this, a dilution series of cell lysate with a methanol/water (8:2, v/v) mixture was performed, yielding a sample range from 250,000 to 5000 HepG2 cell lysates per 10 μL. Samples were prepared using the optimized conditions for TmAmPBr derivatization, after which derivatives were analyzed by CE-MS. In Figure 4, extracted ion electropherograms obtained by CE-MS analysis of carboxylic acid metabolites after derivatization of an extract with a metabolite content equivalent to 25,000 HepG2 cell lysates are shown. Nine of the 11 model compounds could still be detected in a cell extract with an amount of 25,000 cell lysates, where only the content that corresponds to ~70 cell lysates was injected into the capillary. Figure 5

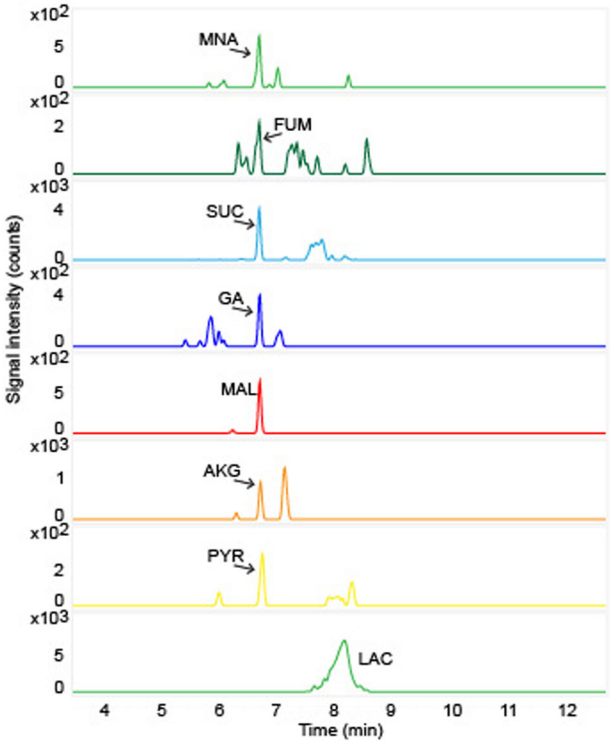

FIGURE 4 Extracted-ion electropherograms obtained by CE-MS from the analysis of 25000 HepG2 cell lysates after derivatization with TmAmPBr. Separation conditions: BGE, 10% (v/v) acetic acid (1.7 M); sample injection volume 27 nL

shows that a linear response was obtained for the endogenous carboxylic acid concentrations when increasing cell numbers from 5000 to 250,000 except for citric acids and oxaloacetic acid, which could only be detected in 250,000 and 100,000 HepG2 cell lysates. Repeatability of the derivatization procedure for carboxylic acid metabolites

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

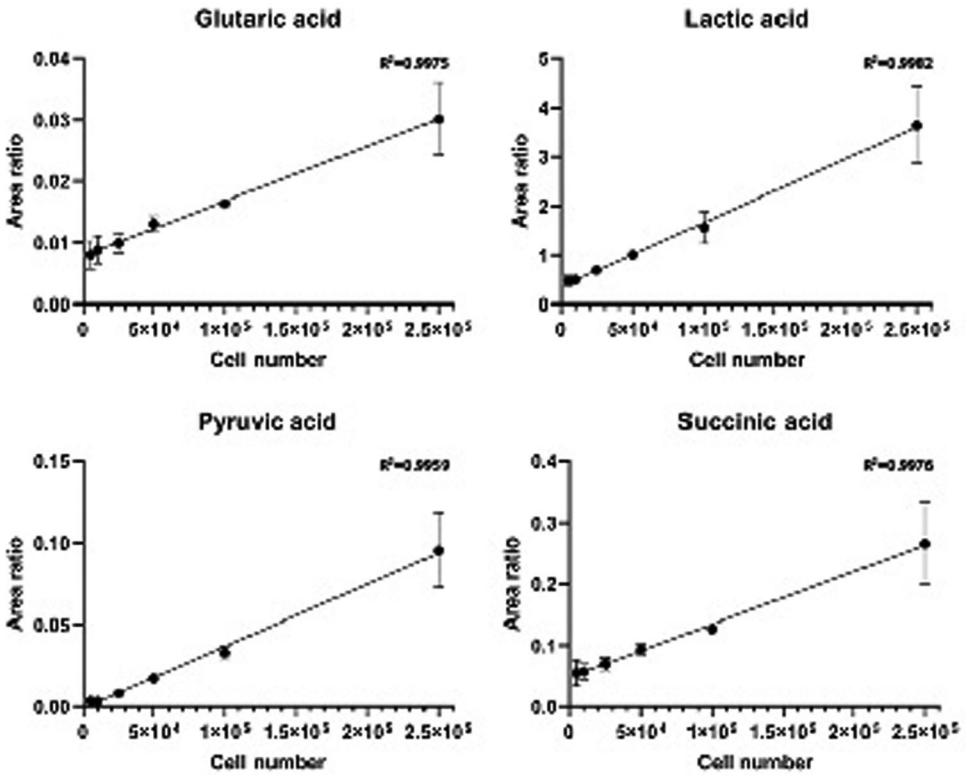

FIGURE 5 Glutaric acid, lactic acid, pyruvic acid, and succinic acid peak area ratios in HepG2 cells (n = 3) ranging from 5000 to 2.5 × 10^5 cells

was assessed for replicates based on the CE-MS analyses of extracts at two concentration levels (250,000 cell lysates and 25,000 cell lysates). Intraday RSD values (n = 3) for peak areas of all analytes were better than 26.4% and 26.9% for 25,000 cell lysates and 250,000 cell lysates, respectively. For the acids that show both single and double derivatized species, the ratio between the mono- and di-form was also investigated in the matrix (250,000 cell lysates), and showed to be stable.

Matrix effect was evaluated by comparing the response of SIL-internal standard succinic acid-d6 in standard solution to its response in a derivatized matrix. MEF was calculated and used to quantitatively evaluate the matrix effect for each cell calibration sample (ranging from 5000 to 2.5 × 10^5 cell lysates),[40] and was between -0.4% and -0.7%. Table 2 gives an overview of the compounds detected in a HepG2 cell extract sample equivalent to 250,000 cell lysates (corresponding to ~700 cell lysates injected) by CE–MS.

It was investigated whether TmAmPBr could be used for the derivatization of other compounds besides carboxylic acid containing metabolites by extracting additional features using the data obtained for 250,000 HepG2 cell lysates as starting material. The features were provisionally annotated using theoretical *m/z* values based on expected labeling patterns.[21] For amino acids such as asparagine, aspartic acid, gamma-aminobutyric acid, glutamine, glutamic acid, glycine, proline, serine, and valine, only a signal was observed for the theoretically determined *m/z* value, and no signal was observed for *m/z* value corresponding to the non-derivatized species. Therefore, it is expected that besides carboxylic acid groups, also other functional groups (such as amine and thiol groups) could be derivatized using TmAmPBr (Supporting Information S11). However, verification using metabolite standards and further examination of the derivatization labeling patterns is needed in order to improve the ability to simultaneously analyze cations and anions in a single CE-MS run.

4 | CONCLUDING REMARKS

In this work, we have proposed a novel chemical derivatization approach that enables the analysis of acidic metabolites by CE-MS by using exactly the same conditions as employed for the analysis of basic metabolites. TmAmPBr derivatization of metabolites containing carboxylic acid groups was optimized using RSM, allowing for multivariate and systematic optimization. The overall method could be used for the quantification of selected acidic metabolites in extracts from 250,000 HepG2 cell lysates, which corresponds to the amount of about 700 cell lysates injected into the capillary. However, to allow the reliable quantification of a broad range of metabolites, the electrophoretic separation needs to be improved further by using a longer capillary. Moreover, we will investigate the use of SIL internal standards for every metabolite, by using an ICD approach during derivatization.[25] The novel derivatization procedure can be further expanded to a wider selection of metabolites, allowing for the analysis of both acidic and basic metabolites in a single CE-MS run using the same separation conditions. Before that, further evaluation of labeling patterns and poten-

Research Article
doi.org/10.1002/ansa.202100054
Chemistry Europe
European Chemical Societies Publishing

TABLE 2 Concentrations determined for carboxylic acid metabolites in extracts from 250.000 HepG2 cell lysates (corresponding to ~700 cell lysates injected) by CE-MS

	Concentration (μM) ± std in 250,000 cells (n = 3)	Repeatability (n = 3) in 250,000 cells	Repeatability (n = 3) in 25,000 cells
Pyruvic acid	7.4 ± 1.3	19.4	26.4
Fumaric acid	3.6 ± 0.1	11.9** (n = 2)	16.6
Glutaric acid	2.8 ± 0.1	15.8	13.1
Malic acid	15± 2.0	15.4	3.8
Succinic acid	7.3 ± 0.9	20.3	12.3
Lactic acid	105 ± 20	17.5	4.9
Oxaloacetic acid	8.8 ± 6.3	25.1** (n = 2)	<LLOQ
Malonic acid	55 ± 9.5	17.1	21.8
a-Ketoglutaric acid	7.8 ± 2.7	15.0** (n = 2)	6.4
Citric acids*	5.1 ± 0.2	26.9	<LLOQ

*Isocitric and citric acid (di) were not baseline separated and integrated as one peak.
**Denotes outlier removal from a single point (Dixon test for outliers at 99% CI, two-tailed test, ©XLSTAT).

tial competition between functional groups (such as amine and thiol groups) when present in the sample matrix is essential. As the focus in the current work was mainly on assessing the utility of the new derivatization agent for analysis of acidic metabolites, limited attention has been paid to improving the resolution of the electrophoretic separation of the acidic metabolites. The resolution could be improved by the use of longer capillaries (obviously resulting in longer separation times) and/or by the use of CE coupled to ion mobility MS (CE-IM-MS) to improve separation between analytes with very similar electrophoretic mobilities.[28] Such aspects will be considered before applying the overall method to the study of biomedical/clinical questions. Moreover, mobility markers procaine and paracetamol will be added after quenching the derivatization procedure in order to be able to determine the electrophoretic mobility, which could significantly aid the identification process in metabolomics, as shown in our recent Metabo-ring study.[11] As the compounds obtain a permanent positive charge after derivatization, it would be interesting to assess the use of electrokinetic injection and also the use of electroextraction for further improving the detection limits.[41]

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGMENTS

M. van Mever and Dr. R. Ramautar acknowledge the financial support of the Vidi grant scheme of the Netherlands Organization of Scientific Research (NWO Vidi 723.016.003). Dr. N. Drouin acknowledges the financial support from Horizon 2020 Marie Sklodowska-Curie COFUND (Grant Agreement No: 707404).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

1. Markley JL, Brüschweiler R, Edison AS, et al. The future of NMR-based metabolomics. *Current Opin Biotechnol*. 2017; 43: 34-40.
2. Aszyk J, Byliński H, Namieśnik J, Kot-Wasik A. Main strategies, analytical trends and challenges in LC-MS and ambient mass spectrometry–based metabolomics. *TrAC Trends Anal Chem*. 2018; 108: 278-295.
3. Beale DJ, Pinu FR, Kouremenos KA, et al. Review of recent developments in GC-MS approaches to metabolomics-based research. *Metabolomics*. 2018; 14: 1-31.
4. Segers K, Declerck S, Mangelings D, Vander Heyden Y, Van Eeckhaut A. Analytical techniques for metabolomic studies: a review. *Bioanalysis*. 2019; 11: 2297.
5. Zhang W, Ramautar R. CE-MS for metabolomics: developments and applications in the period 2018-2020. *Electrophoresis*. 2021; 42: 381-401.
6. Ishibashi Y, Harada S, Takeuchi A, et al. Reliability of urinary charged metabolite concentrations in a large-scale cohort study using capillary electrophoresis-mass spectrometry. *Sci Rep*. 2021; 11: 7407-7407.
7. Lombard-Banek C, Li J, Portero EP, et al. *Angew Chem Int Ed*. 2021.
8. van Mever M, Segers K, Drouin N, et al. Direct profiling of endogenous metabolites in rat brain microdialysis samples by capillary electrophoresis-mass spectrometry with on-line preconcentration. *Microchem J*. 2020; 156.
9. Garcia A, Godzien J, Lopez-Gonzalvez A, Barbas C. Capillary electrophoresis mass spectrometry as a tool for untargeted metabolomics. *Bioanalysis*. 2017; 9: 99-130.
10. Harada S, Hirayama A, Chan Q, et al. Reliability of plasma polar metabolite concentrations in a large-scale cohort study using capillary electrophoresis-mass spectrometry. *PLoS One*. 2018; 13: e0191230.
11. Drouin N, van Mever M, Zhang W, et al. Capillary electrophoresis-mass spectrometry at trial by metabo-ring: effective electrophoretic mobility for reproducible and robust compound annotation. *Anal Chem*. 2020; 92: 14103-14112.
12. van Mever M, Hankemeier T, Ramautar R. CE-MS for anionic metabolic profiling: an overview of methodological developments. *Electrophoresis*. 2019;40;2349-2359.
13. Soga T, Ueno Y, Naraoka H, Ohashi Y, Tomita M, Nishioka T. Simultaneous determination of anionic intermediates for bacillussubtilis metabolic pathways by capillary electrophoresis electrospray ionization mass spectrometry. *Anal Chem*. 2002;74:2233.

14. Soga T, Igarashi K, Ito C, Mizobuchi K, Zimmermann H-P, Tomita M. Metabolomic profiling of anionic metabolites by capillary electrophoresis mass spectrometry. *Anal Chem*. 2009; 81: 6165.
15. Buscher JM, Czernik D, Ewald JC, Sauer U, Zamboni N. Cross-platform comparison of methods for quantitative metabolomics of primary metabolism. *Anal Chem*. 2009; 81: 2135.
16. Yamamoto M, Ly R, Gill B, Zhu Y, Moran-Mirabal J, Britz-Mckibbin P. Robust and high-throughput method for anionic metabolite profiling: preventing polyimide aminolysis and capillary breakages under alkaline conditions in capillary electrophoresis-mass spectrometry. *Anal Chem*. 2016; 88: 10710.
17. Lee J-H, Kim S-J, Lee S, Rhee J-K, Lee SY, Na Y-C. Saturated fatty acid determination method using paired ion electrospray ionization mass spectrometry coupled with capillary electrophoresis. *Anal Chim Acta*. 2017;984:223-231.
18. Zhang W, Guled F, Hankemeier T, Ramautar R. Profiling nucleotides in low numbers of mammalian cells by sheathless CE-MS in positive ion mode: circumventing corona discharge. *Electrophoresis*. 2020;40:360-369.
19. Drouin N, Pezzatti J, Gagnebin Y, González-Ruiz V, Schappler J, Rudaz S. Effective mobility as a robust criterion for compound annotation and identification in metabolomics: toward a mobility-based library. *Anal Chim Acta*. 2018;1032:178-187.
20. Wuethrich A, Quirino JP. Derivatisation for separation and detection in capillary electrophoresis (2015-2017). *Electrophoresis*. 2018;39:82-96.
21. Willacey CCW, Naaktgeboren M, Lucumi Moreno E, et al. LC-MS/MS analysis of the central energy and carbon metabolites in biological samples following derivatization by dimethylaminophenacyl bromide. *J Chromatogr A*. 2019: 460413.
22. Denver N, Khan S, Stasinopoulos I, et al. Derivatization enhances analysis of estrogens and their bioactive metabolites in human plasma by liquid chromatography tandem mass spectrometry. *Anal Chim Acta*. 2019;1054:84-94.
23. Wong JM, Malec PA, Mabrouk OS, Ro J, Dus M, Kennedy RT. Benzoyl chloride derivatization with liquid chromatography-mass spectrometry for targeted metabolomics of neurochemicals in biological samples. *J Chromatogr A*. 2016;1446:78-90.
24. Song P, Mabrouk OS, Hershey ND, Kennedy RT. In vivo neurochemical monitoring using benzoyl chloride derivatization and liquid chromatography-mass spectrometry. *Anal Chem*. 2012;84: 412-419.
25. Willacey CC, Karu N, Harms AC, Hankemeier T. Metabolic profiling of material-limited cell samples by dimethylaminophenacyl bromide derivatization with UPLC-MS/MS analysis. *Microchem J*. 2020:159:105445.
26. Guo K, Li L. Differential 12 C-/ 13 C-isotope dansylation labeling and fast liquid chromatography/mass spectrometry for absolute and relative quantification of the metabolome. *Anal Chem*. 2009;81:3919-3932.
27. Luo X, Li L. Metabolomics of small numbers of cells: metabolomic profiling of 100, 1000, and 10000 human breast cancer cells. *Anal Chem*. 2017; 89: 11664-11671.
28. Yang WC, Regnier FE, Adamec J. Comparative metabolite profiling of carboxylic acids in rat urine by CE-ESI MS/MS through positively precharged and 2 H-coded derivatization. *Electrophoresis*. 2008;29:4549-4560.
29. D'Agostino LA, Lam KP, Lee R, Britz-McKibbin P. Comprehensive plasma thiol redox status determination for metabolomics. *J Proteome Res*. 2011;10:592-603.
30. Huang T, Armbruster M, Lee R, Hui DS, Edwards JL. Metabolomic analysis of mammalian cells and human tissue through one-pot two stage derivatizations using sheathless capillary electrophoresis-electrospray ionization-mass spectrometry. *J Chromatogr A*. 2018; 1567: 219-225.
31. Guo K, Li L. High-performance isotope labeling for profiling carboxylic acid-containing metabolites in biofluids by mass spectrometry. *Anal Chem*. 2010;82:8789-8793.
32. Drouin N, Mielcarek A, Wenz C, Rudaz S. Evaluation of ion mobility in capillary electrophoresis coupled to mass spectrometry for the identification in metabolomics. *Electrophoresis*. 2021;42:342-349.
33. Wong J-MT, Malec PA, Mabrouk OS, Ro J, Dus M, Kennedy RT. Benzoyl chloride derivatization with liquid chromatography-mass spectrometry for targeted metabolomics of neurochemicals in biological samples. *J Chromatogr A*. 2016;1446:78-90.
34. Marquis BJ, Louks HP, Bose C, Wolfe RR, Singh SP. A new derivatization reagent for HPLC-MS analysis of biological organic acids. *Chromatographia*. 2017;80:1723-1732.
35. Yang WC, Regnier FE, Adamec J. Comparative metabolite profiling of carboxylic acids in rat urine by CE-ESI MS/MS through positively precharged and 2 H-coded derivatization. *Electrophoresis*. 2008;29:4549-4560.
36. Lord, H & Pfannkoch, EA. Sample Preparation Automation for GC Injection. Comprehensive Sampling and Sample Preparation. Elsevier Inc. 2012; 2: 597-612.
37. Guo K, Li L. High-performance isotope labeling for profiling carboxylic acid-containing metabolites in biofluids by mass spectrometry. *Anal Chem*. 2010;82:8789-8793.
38. Derringer G, Suich R. Simultaneous optimization of several response variables. *J Qual Technol*. 1980;12:6.
39. Van Loco J, Elskens M, Croux C, Beernaert H. Linearity of calibration curves: use and misuse of the correlation coefficient. *J Qual Compar Reliab Chem Measurement*. 2002; 7: 281-285.
40. Zhou W, Yang S, Wang PG. Matrix effects and application of matrix effect factor. *Bioanalysis*. 2017; 9: 1839-1844.
41. He Y, Miggiels P, Wouters B, et al. A high-throughput, ultrafast, and online three-phase electro-extraction method for analysis of trace level pharmaceuticals. *Anal Chim Acta*. 2021;1149:338204-338204.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: van Mever M, Willacey CCW, Zhang W, et al. Profiling acidic metabolites by capillary electrophoresis-mass spectrometry in low numbers of mammalian cells using a novel chemical derivatization approach. *Anal Sci Adv*. 2021;1-11. https://doi.org/10.1002/ansa.202100054