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The role of capillary electrophoresis in metabolic profiling studies employing multiple analytical techniques

Miranda G.M. Kok [a,b,*], Govert W. Somsen [c], Gerhardus J. de Jong [a,b]

[a] Biomolecular Analysis, Department of Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands
[b] Research Group Analysis Techniques in the Life Sciences, Avans Hogeschool, P.O. Box 90116, 4800 RA Breda, The Netherlands
[c] AIMMS Division of BioAnalytical Chemistry, Faculty of Sciences, VU University, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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ABSTRACT

Capillary electrophoresis-mass spectrometry (CE-MS) is increasingly used for the targeted and untargeted analysis of metabolites in biological samples. CE-MS is particularly useful for the profiling of highly polar metabolites without the need for derivatization and/or extensive sample preparation. This overview covers reported studies in which CE or CE-MS was used with direct MS, gas chromatography (GC)-MS, liquid chromatography (LC)-MS, and/or nuclear magnetic resonance (NMR) spectroscopy for the analysis of metabolites. We include an extensive overview of comparative metabolic profiling studies involving multiple analytical techniques, including CE. Tables set out the particular conditions and the characteristics of each study. Furthermore, we discuss and compare the results of these studies using selected examples. Based on the outcomes of the comparative studies, we conclude that CE is highly complementary to the other analytical technologies and can add essential information on the metabolic profile of biological samples.

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Abbreviations: BGE, Background electrolyte; CE, Capillary electrophoresis; EI, Electron ionization; EOF, Electroosmotic flow; ESI, Electrospray ionization; FA, Formic acid; FT-ICR, Fourier transform-ion cyclotron resonance; GC, Gas chromatography; HILIC, Hydrophilic interaction chromatography; LC, Liquid chromatography; LOD, Limit of detection; MALDI, Matrix-assisted laser desorption/ionization; MEKC, Micellar electrokinetic chromatography; MS, Mass spectrometry; MSTFA, N-methyl-N-(trimethylsilyl)trifluoroacetamide; NMR, Nuclear magnetic resonance; PCA, Principal component analysis; PLS-DA, Partial least squares discriminant analysis; QC, Quality control; RP, Reversed phase; SDS, Sodium dodecyl sulfate; TBDMS-FA, N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide; TCA, Tricarboxylic acid; TMCS, Trimethylchlorosilane; (Q)-TOF, (Quadrupole) time-of-flight; TSP, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid; UP, Ultra-performance; UV, Ultraviolet.

* Corresponding author. Tel.: +31 302537307; Fax: +31 302536655.
E-mail address: m.g.m.kok@uu.nl (M.G.M. Kok).

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1. Introduction

Metabolomics encompasses the identification and the quantification of low-molecular-weight endogenous compounds in biological samples, such as cells, tissues and body fluids. Metabolomics provides information on biochemical processes and phenotypes of organisms, and can be applied using a targeted or an untargeted strategy. In a targeted approach, specific (classes of) metabolites are analyzed with the aim of determining differences in their concentration between samples. In an untargeted approach, global profiling of as many metabolites as possible is attempted in order to reveal new potential biomarkers [1–4]. Global metabolic profiling is challenging, since metabolites have very diverse physicochemical properties and can occur in a broad concentration range in biological systems. In recent years, many analytical techniques have been developed to profile metabolites in biological samples [5]. Ideally, the analytical techniques are rapid, sensitive and reproducible, requiring no or simple sample preparation, and being capable of analyzing nearly all metabolites. Not surprisingly, no one analytical technique meets all these requirements.

Nuclear magnetic resonance (NMR) spectroscopy is most commonly used in metabolic profiling studies, especially ^{1}H NMR spectroscopy, which is a rapid, non-destructive analytical technology that provides information on the identity and the concentration of metabolites, without the need for prior separation. A drawback is its limited sensitivity.

Mass spectrometry (MS) detection is highly sensitive and provides information on metabolite masses. When mass spectrometers with a high mass accuracy and resolution are used, putative identification based on accurate mass might be possible. MS can be used as a stand-alone system in so-called flow-injection mode, but it is often coupled to separation techniques in order to distinguish isobaric compounds and to reduce sample matrix effects (e.g., ion suppression) [6].

Gas chromatography (GC) and liquid chromatography (LC) are two chromatographic separation techniques that are often combined with MS. In GC, metabolites are brought in the gas phase and separated with high resolution based on interaction with a liquid stationary phase. For GC analysis, compounds need to be volatile and that can be achieved using pre-column chemical derivatization procedures, which are time consuming and labor intensive and may introduce artifacts (e.g., incomplete derivatization) [7]. The basic separation principle in LC is the difference in partition of compounds between a mobile liquid phase and a stationary phase. Different separation modes exist, of which reversed-phase (RP) LC is most often applied in metabolomics studies. RPLC is not well suited to the analysis of highly polar and charged compounds, which elute in the column dead time. Hydrophilic interaction chromatography (HILIC) has been introduced for the profiling of these polar compounds, providing complementary metabolite information to RPLC. In HILIC, besides partitioning between the mobile phase and a stagnant water layer on the surface of the stationary phase, compounds are also retained by electrostatic interactions with the polar stationary phase employed [8,9].

The various analytical techniques are most suited to different (classes of) metabolites, so platforms applying them have been employed to obtain comprehensive coverage of the metabolome and to compare the outcomes and the characteristics of the techniques. Recently, capillary electrophoresis (CE) was added to the analytical toolbox for metabolomics and studies that involved CE next to other analytical techniques were carried out. CE-MS was introduced as a powerful technique for the profiling of highly polar and charged metabolites. Compounds are separated based on their charge-to-size ratio and only very small amounts of sample and separation electrolytes are required [10]. CE-MS has the potential to provide complementary information to other more common methodologies, thereby increasing metabolite coverage. In order to evaluate the contribution and the added value of CE in metabolomics, this article gives an overview of metabolic profiling studies, involving CE and one or more other analytical techniques, which were reported by December 2013 [11–59].

In some studies, CE was compared with other analytical technologies, whereas, in other reported studies, CE and the other techniques were applied to analyze different metabolite classes and to obtain a comprehensive coverage of the metabolome.

The review starts with a discussion on general aspects of the techniques applied in the reported comparative metabolic profiling studies, including analytical set-up, sample preparation, MS detection and data analysis. In the second part, we give a detailed overview of studies comparing multiple analytical techniques including CE. Tables summarize characteristics of each study. Moreover, we discuss and give examples of results of the reported studies. We briefly cover the non-comparative studies to consider which classes of metabolites can be efficiently analyzed with CE and the other applied methodologies.

2. General aspects of analytical methodologies used in comparative metabolic profiling studies

A typical metabolic profiling study involves multiple steps, as depicted in Fig. 1. After appropriate experimental design, sample collection and preparation, the samples are analyzed with one or more analytical methodologies. Acquired data are analyzed after several preprocessing steps, such as alignment, normalization and scaling. Multivariate data analysis is applied to determine differences in metabolite concentrations between different groups of samples. In the final steps, identification of metabolites of interest is pursued and their role in biochemical pathways is interpreted.

We discuss below general aspects of the analytical techniques used in comparative metabolic profiling studies.

2.1. Methods

Predominantly, three CE methods have been used in comparative profiling studies to separate cationic, anionic and neutral metabolites. Cationic compounds have been analyzed using a background electrolyte (BGE) containing formic acid (FA) with a pH of approximately 2 [12,15,18,20,23–25,28,43–45]. Anionic metabolites are most often separated in a BGE containing ammonium acetate, but ammonium carbonate and formate have also been used. The pH of the employed BGEs with ammonium salts varied in the range 7.5–10.0 [11–14,22,26,43,45]. Neutral metabolites have been analyzed with (micellar) electrokinetic chromatography ((M)EKC) using sodium dodecyl sulfate (SDS) and sulfated β-cyclodextrin [19,21,27,53,54]. Separations have usually been carried out in bare fused-silica capillaries [12,15,18–23,25–28,43–45,53–55], although coated capillaries have been applied in some studies [11,13,14,19,24,43,45,53,54]. The use of capillaries coated with positively charged polymers reverses the electroosmotic flow (EOF), and, hence, the migration order of the metabolites. Furthermore, coated capillaries are applied to diminish adsorption of matrix components to the inner wall of the capillary, resulting in a more reproducible EOF and analyte migration times required for reliable comparisons of obtained metabolite profiles [60,61].

Sample pretreatment may be necessary for good analytical CE performance. In global metabolic profiling studies, minimal sample preparation is desirable to prevent metabolite losses. The sample pretreatment depends on the nature of the samples. Types of sample that have been analyzed in comparative metabolic profiling studies involving CE are urine, plasma and extracts of bacteria, cells, plants and tissues.

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