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ELECTROPHORESIS

RESEARCH ARTICLE

# In-line sample trap columns with diatomite for large-volume injection in CZE–IM–MS

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## Abstract

The analysis of low-abundant compounds with capillary zone electrophoresis–drift-tube ion mobility spectrometry–mass spectrometry (CZE–DTIMS–MS) is compromised due to the low injectable sample volumes in CZE and low duty cycle in DTIMS. Fritless packed in-line trap columns, using solid-phase extraction sorbent particles, have been used to increase injection volumes in CZE, but these columns are difficult to prepare and exhibit rapidly increasing back pressures. To provide smooth and complete filling of trap columns as well as to ensure higher and sustained flow rates though the columns, blends of cation and anion exchange particles with diatomite were used. The application of diatomite blends ensured the use of trap columns for at least 100 injections, with maximum injection volumes over 10 µl, which corresponds to an enrichment factor of more than 1000 over conventional injections in CZE–MS, enabling the detection of low nM concentrations of *N*-glycans with CZE–IMS–MS.

**KEYWORDS**
capillary electrophoresis, glycans, ion mobility spectrometry, mass spectrometry, solid-phase extraction

## 1 | INTRODUCTION

Capillary zone electrophoresis (CZE) techniques have played an important role in developments in the life sciences, providing an efficient separation of small polar metabolites as well as of larger biomolecules, such as DNA, proteins, and glycans [1–4]. The coupling with mass spectrometry (MS) has ensured a broader application of CZE in the life sciences, offering additional structural information, such as the determination of glycan compositions using accurate mass measurements and glycan linkages through multistage MS measurements [3, 5]. More recently, ion mobility spectrometry (IMS)–MS has been introduced for comprehensive glycan analysis, facilitating isomeric glycan separation and characterization by collision cross-section (CCS) values as well as by specific arrival time distributions (ATDs) [6–9]. The coupling of CZE with IMS–MS creates a synergy in which

**Abbreviations:** ATD, arrival time distribution; CCS, collision cross section; DTIMS, drift-tube ion mobility spectrometry; DVB-NVP, *m*-divinylbenzene and *N*-vinylpyrrolidone copolymer; FA, formic acid; FC, fluorocarbon; G2, di-galactosylated *N*-glycan; G7, maltoheptaose; IMS, ion mobility spectrometry; LE, leading electrolyte; MAX, strong anion exchange resin; MCX, strong cation exchange resin; PA, procainamide; TE, terminating electrolyte; WAX, weak anion exchange resin; WCX, weak cation exchange resin.

efficient separations with increased selectivity can be obtained with identification based on mass, CCS value, and ATDs [9, 10]. On the other hand, a CZE–IMS–MS system also compromises detection sensitivity due to a lowered duty cycle in IMS–MS, in particular with drift-tube (DT)IMS–MS, whereas the sensitivity with CZE–MS is already lower compared to liquid chromatography (LC)–MS methods due to the low nl injection volumes used in CZE [2, 7]. The application of larger injection volumes in CZE could overcome the sensitivity problems, but injection volumes in CZE are usually limited to 1%–2% of the capillary volume to ensure field-amplified sample stacking, which provides the typical efficient electrophoretic peaks [11]. To inject larger volumes while maintaining efficient peaks, several high-volume injection techniques can be applied, based on stacking [11] or chromatographic techniques [12]. Stacking techniques, such as large-volume sample stacking, offer a limited enrichment factor and often require an outlet background electrolyte (BGE) vial, which is absent in a conventional CZE–MS setup. Chromatographic fritless in-line solid-phase extraction (SPE) trap columns with packed polymeric sorbent beds have been used to obtain sample enrichment [13–15] and are easier implementable with CZE–MS. Several microliters of sample can be injected, depending on the capacity of the column, which leads to preconcentration factors of more than 100. Fritless trap columns contain particles that are larger in diameter than the preceding inlet capillary and the outlet separation capillary, which ensure that particles remain trapped in the columns during analysis [14]. However, the filling of these trap columns is difficult due to caking and wedging of particles, leading to hollow spaces and irreproducible as well as incomplete filling of the trap column. Furthermore, the formation of a dense cake and compression of particles increases back pressure over time, which leads to irreproducible results, electric current breakdowns, impermeable columns at the low pressures used in capillary electrophoresis (CE), and short trap column durability [13]. The low robustness of SPE–CZE so far has ensured that this approach has yet not been applied routinely.

Here we show in-line sample trapping for CE, using robust trap columns with commercially available polymeric SPE particle sorbents, blended with diatomite. Diatomite, also known as diatomaceous earth, Celite, or kieselguhr consist of natural amorphous silica formed from fossils of unicellular organisms that form intricately shaped and highly porous particles. It is used as a filter aid to create a highly permeable and incompressible layer in filters [16], as a solid support in automated liquid–liquid extraction [17], and it played an important role in the development of stationary phases for gas chromatography and LC [18–20]. It is also used as an anticaking agent and glidant in the pharmaceutical and food/feed industry to improve the flow of solid powders [21]. The combination of diatomite with several types of polymeric SPE particles allowed for smooth and consistent filling of trap columns, and for the formation of an incompressible and permeable chromatographic cake, which allows sustained low-pressure-driven flow rates for large-volume injections in CZE. The trap columns, with cation or anion exchange particles or combinations thereof, were applied to enrich samples for *N*-glycan analysis with CZE–DTIMS–MS using a conventional and robust CE–MS sheath liquid sprayer interface. Trap columns were used for up to 340 sample runs, using several microliter volume injections, without affecting their characteristics and performance, which demonstrates the robustness that is necessary for routine CZE–MS analysis.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals

Methanol (MeOH; LC–MS grade) and acetonitrile (ACN) (LC–MS grade) were obtained from Biosolve (Valkenswaard, the Netherlands). Ultrapure water was generated by a Synergy UV water purification system (Burlington, MA, USA), and Oasis SPE sorbents were obtained from Waters (Milford, MA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-glycan standards were synthesized in-house by a chemoenzymatic approach [8].

### 2.2 | Glycan labeling

*N*-Glycans were labeled by adding 10 µl water and 10 µl labeling mixture, consisting of 50 mg/ml 4-amino-*N*-2-(diethylamino)ethyl-benzamide (procainamide, PA) or other derivatization agents and, if necessary, 50 mg/ml sodium cyanoborohydride in 10:3 (v/v) DMSO/acetic acid for reduction. This solution was incubated at 65°C for 2 h and cleaned up using a previously described porous graphitized carbon SPE method [8]. The sample was dried under a nitrogen stream and reconstituted in 20 µl water before analysis. The derivatization procedure was also applied to glycan standards, using different derivatization agents.

### 2.3 | Preparation of trap columns

Diatomite (Celite 545, 20–100 µm particles; Merck KGaA, Darmstadt, Germany) and both strong and weak anion and cation exchange Oasis SPE particles (~60 µm) were

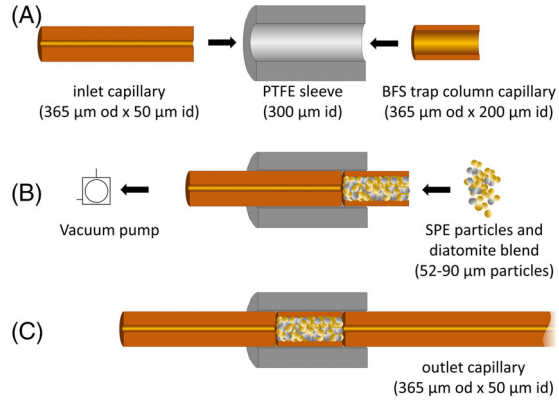


**FIGURE 1** Schematic overview of the construction of the trap column: (A) cross sections of bare fused silica (BFS) capillaries in a polytetrafluoroethylene (PTFE) sleeve. The capillaries have an outer diameter (od) that matches the inner diameter (id) of the sleeve. (B) The capillaries are inserted into the sleeve and thereafter sorbent particles, with a larger diameter than the in- and outlet capillaries, are introduced into the solid-phase extraction (SPE) capillary by applying a vacuum through the inlet capillary. (C) The packed SPE capillary is pushed to the center of the sleeve with a BFS capillary, and the outlet capillary is connected.

extensively dried at 65°C and sieved through steel powder sieves to obtain only dry particles ranging from 52 to 90 µm. This procedure was repeated several times until all diatomite and SPE particles <52 µm were removed. Diatomite and SPE particles were mixed in different ratios and dry packed in 4 mm long bare fused silica (BFS) capillaries (200 µm id, 365 µm od, Biotaq, Gaithersburg, MD, USA) by applying vacuum on one side of the capillary [14]. The combined capillary was assembled by connecting one side of the trap column to a 10 cm, 50 µm id inlet and the other side to a 60–100 cm, 50 µm id outlet BFS capillary through a sleeve to ensure a tight connection (Figure 1) [14]. The sleeve consisted of a 1.5 cm long, 1.58 mm od, and 0.3 mm id Teflon tube (Sigma-Aldrich). The id of the sleeve was slightly increased by first passing through a 10 cm long, 365 µm od BFS capillary to allow capillaries to be connected more easily and to prevent the scraping of Teflon pieces of the inside of the sleeve, which could clog the combined BFS capillary during assembly. Besides uncoated BFS capillaries, in- and outlet capillaries with fluorocarbon (FC) polymer coating (Agilent Technologies, Santa Clara, CA, USA) were used for CZE–IMS–MS.

The sample trapping on the trap columns was performed with four different polymeric SPE materials (*m*-divinylbenzene and *N*-vinylpyrrolidone copolymer [DVB–NVP] with different substituents) and chemoenzymatically synthesized *N*-glycan standards, with chargeable anomeric tags attached through reductive amination. Four different tags with corresponding sorbents were initially used (Table S1): Girard's reagent-T tag in combination with weak cation exchange resin (WCX; containing carboxylic acid substituents), PA tag with strong cation exchange resin (MCX; with sulfonic acid substituents), 2-aminobenzoic acid tag with strong anion exchange resin (MAX; with quaternary amine substituents), and aminopyrene trisulfonate tag with weak anion exchange resin (WAX; modified with piperazine substituents).

## 2.4 | CE–IMS–MS system

The capillaries with SPE trap columns were used in a 7100 CE instrument and connected to a 6560 DTIMS-mass spectrometer through a coaxial sheath liquid-jet stream electrospray interface (Agilent Technologies), using an optimized sheath liquid of MeOH:water 70:30 (v/v) with 20 mM formic acid (FA) at a flow rate of 5 µl/min. The optimal MS interface conditions were a capillary voltage of 1725 V, a drying gas temperature of 250°C, a drying gas flow rate of 3 L/min, a nebulizer pressure of 6 psi, a sheath gas temperature of 195°C, and a sheath gas flow of 3.5 L/min.

Instrument settings for IMS and CZE were optimized with PA-labeled *N*-glycans for optimal signal response. For CZE, the BGE composition and pH were optimized in combination with the elution solvent for the trap column as these solvents affected both CZE and elution of *N*-glycans. Flushing times of these solvents at specific pressures were dependent on the flow rate through the capillary and trap column, which was determined daily by flushing an aqueous solution of glucose through the capillary at a specific pressure and by determining the arrival time at the CE capillary exit, detected by MS. The consecutive volumes of optimized flushing solutions, with an outlet capillary of 88 cm, were three capillary volumes (6 µl) 50 mM FA to condition the trap column, up to 6.8 capillary volumes sample injection (13.6 µl), one capillary volume (1.5 µl) 50 mM FA, two trap column bed volumes (175 nl, assuming a sorbent column occupancy of 70%) of eluent, and 2 M acetic acid (HAc, 290 nl) to push the eluent plug through the trap column. The elution plug was moved back and forth several times through the trap column to ensure an efficient desorption and then pushed into the separation capillary with 250 nl 2 M HAc for CE separation. For outlet capillaries with a different length, the flushing volumes for the BGE were adjusted to displace equal capillary volumes of solvents. Separations were performed at 25°C with a separation voltage of 30 kV, with both FC-coated and FC-uncoated BFS capillaries.

IMS separation was performed in an 80 cm drift tube, operated at an entrance voltage of 1700 V and exit voltage of 250 V, using nitrogen as drift gas. The trap funnel pressure was maintained at 3.85 Torr and drift-tube pressure

at 4.00 Torr. Maximum drift time was set at 60 ms, with a trapping time of 20 ms and a release time of 150 µs.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Trap column development

Trap columns were filled with blends of MCX, WCX, MAX, or WAX Oasis SPE particles with diatomite. These columns have a reduced capacity, compared to columns with pure SPE sorbents, and to obtain a sufficient sorbent surface area, trap columns with a length of 4 mm and 200 µm ID were used, which are larger than in previously optimized in-line SPE-CE systems [14, 15].

The powder density of diatomite is approximately half of the powder density of the SPE particles. Given the difficulty to determine accurate powder volumes, the corresponding amounts of diatomite and SPE particles were determined by weight to obtain repeatable blends. To establish the optimal diatomite:SPE particle sorbent ratio, diatomite and SPE particles were mixed in ratios ranging from 1:4 to 4:1 (m/m). With a quantity of diatomite in blends below 50% (% m/m), the dry packing of trap columns was difficult, leading to incomplete filling and rapidly increasing back pressures after solvent flushing at a pressure of 1 bar, resulting in impermeable columns. With a 50% (% m/m) or higher diatomite quantity, a complete filling was accomplished within seconds, and the packed columns could be flushed at a pressure of 1 bar, demonstrating the positive effect of diatomite blends on the flow of SPE particles into the trap column and solvent flow through the packed bed. The four different SPE sorbents, containing 50% (% m/m) diatomite, were tested by trapping a di-galactosylated *N*-glycan (G2), containing the corresponding anomeric tag, washing the trap column with water, and eluting with one bed volume (88 nl) of the generally recommended Oasis SPE elution solvents (Table S1), at a pressure of 1 bar. The eluted G2 standard was then pushed further by pressure through the outlet capillary to the MS detector. The four different types of sorbents all successfully retained G2, with the combination of MCX strong cation exchange sorbent and PA tag yielding the highest MS response for G2 at this stage, although none of the trapping systems had been optimized yet. The combination of PA tag with MCX sorbent was used for further optimization and characterization of trap columns with diatomite blends. The PA tag is predominantly protonated due to the presence of a tertiary amine and can be multiply charged under acidic conditions due to the secondary amine linked to the carbohydrate, ensuring sufficient charge for sample trapping, electrophoretic migration, and MS detection.

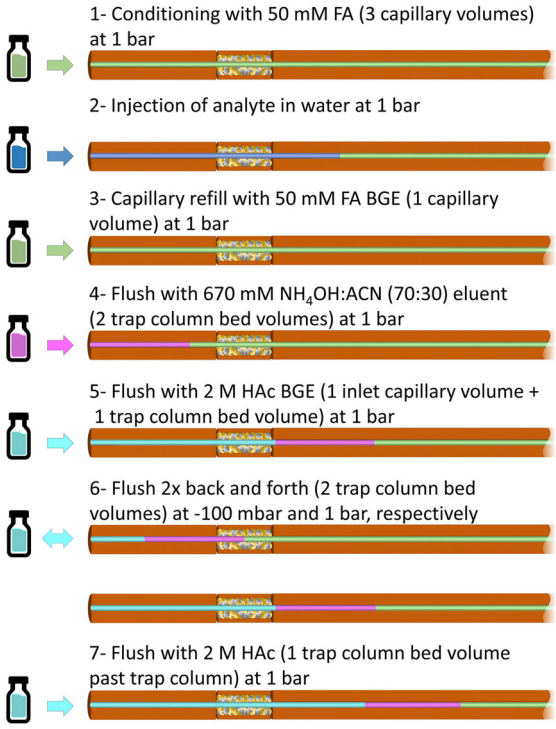


**FIGURE 2** Injection and elution steps for *N*-glycans on a trap column with strong cation exchange resin (MCX) sorbent

### 3.2 | Optimization of trapping, elution, and CZE

#### 3.2.1 | Flow rate

To determine the flow rate through the capillary with trap column at 100 mbar and 1 bar pressures, a 1 mM aqueous solution of unlabeled glucose, which is not retained on the MCX column, was flushed through the capillary and detected by MS. The arrival time at the MS detector was used to calculate the flow rate to adjust pressure application times and ensure accurate flushing, injection, and elution volumes. Where possible, all pressure applications in the method were performed at 1 bar as there was no difference in signal intensity when applying equal volumes during injection and elution steps at 100 mbar or 1 bar (data not shown).

#### 3.2.2 | Trapping and elution

A trap column, connected to a 100 cm BFS outlet capillary, was conditioned with 50 mM FA (Figure 2, step 1), and a 100 nl volume of 6 µM G2-PA was injected hydrodynamically into the column at a pressure of 1 bar (Figure 2, step 2). Injections of G2-PA dissolved in acidified solutions and organic solvents resulted in poor retention, whereas G2-PA was retained on the trap column when water was

used as the solvent. Therefore, all sample injections were performed with water as a solvent.

The elution of G2-PA from the column (Figure 2, step 4) was optimized in three steps by first optimizing the BGE solution composition, which is important to prevent sample breakthrough during filling of the outlet capillary with BGE (Figure 2, step 3). Then the composition of the elution solution was optimized by flushing two bed volumes (175 nl) of different eluent compositions through the trap column, and finally, the optimal elution plug volume was determined by eluting with different bed volumes. After flushing with one capillary volume (2.3 µl) of 2 M FA or 2 M acetic acid (HAc), most of the trapped G2-PA was eluted by these high ionic strength solutions. During flushing with one capillary volume of 10 mM HAc, approximately 50% sample still eluted, probably caused by favorable ion-paring of G2-PA and acetate. On the other hand, all G2-PA was retained on the trap column after flushing with four capillary volumes of up to 100 mM FA. To maintain a safe margin, 50 mM FA was used to fill the separation capillary after sample trapping (Figure 2, step 3). Next, the optimal eluent composition was determined by flushing with different organic solvents in a ratio of 1:1 (v/v) with 5% (% v/v) concentrated ammonium hydroxide (670 mM; Figure 2, step 4). Ammonium hydroxide was selected as it provides a high pH and high ionic strength for the elution of weak cations from the MCX sorbent. Furthermore, ammonium ions can also act as leading electrolyte (LE) in transient isotachophoresis (ITP) in a system with acidic solutions as terminating electrolyte (TE) [22]. Elution was most efficient with ACN as an organic solvent, with a minimum of 10% ACN (% v/v) needed to elute G2-PA, and ≥30% ACN (% v/v) yielding the highest recovery (Figure S1A,B). For further optimization, the ammonium hydroxide concentration was varied in an ACN:water 30:70 (v/v) elution solvent. High concentrations of concentrated ammonium hydroxide (>5% (%v/v)) showed the best elution of G2-PA (Figure S1C), and elution with larger bed volumes (>2) also resulted in higher G2-PA recovery (Figure S1D). To further improve the recovery, while applying practical elution volumes, eluent plugs with different ammonium hydroxide concentrations and bed volumes were passed back and forth several times through the trap column by applying alternating positive and negative pressures on the CE inlet vial (Figure 2, steps 5 and 6). A maximum recovery was obtained by eluting with 5% (% v/v) concentrated ammonium hydroxide (670 mM) in ACN:water 30:70 (v/v), with an elution plug of two bed volumes and by passing the eluent two times back and forth through the trap column. This way, the eluent plug is passed five times through the trap column, which ensures the maximum desorption of G2-PA with a recovery of 87%. The elution plug was finally flushed with one bed volume HAc BGE past the trap column into the outlet capillary to prevent current breakdowns when applying the separation voltage (Figure 2, step 7).

### 3.2.3 | Capillary coating and transient ITP-CZE

When the CE capillary was flushed with eluent, after eluting and forcing G2-PA out of the capillary by pressure, additional peaks of G2-PA eluted out of the CE capillary due to G2-PA adsorption to the BFS capillary wall. Therefore, an FC-coated capillary was used as inlet and outlet capillary to prevent adsorption to the capillary wall of PA-derivatized compounds.

The ammonium ions in the optimized elution plug served as LE for transient ITP, and several volatile BGEs were applied to optimize the TE. FA and HAc at high (2–4 M) and low molarities (10–100 mM) were flushed through the capillary after the eluent plug, to fill the inlet side of the capillary with TE. After passing the eluent back and forth two times, the plug was moved into the outlet capillary, and a 30 kV voltage was applied to start the ITP process. The separation was assisted by pressure to ensure the migration of G2-PA out of the capillary before the transition to CZE. FA as TE resulted in broad peaks for G2-PA in the electropherograms, without effectuating ITP, probably caused by the high mobility of protons in the TE. The protons in HAc have a lower mobility due to the higher buffering capacity of the solution, and the application of this weaker acid resulted in more concentrated analyte zones [22]. All HAc TE solutions effectuated ITP, but low HAc concentrations slowed down the transition from ITP to CZE. A TE of 2 M HAc, in combination with 670 mM ammonium hydroxide as LE, ensured ITP and provided the subsequent separation of G2-PA and free PA with CZE (Figure S2). To demonstrate the requirement of ammonium ions to create a concentrated G2-PA ITP zone, an elution plug with 30% ACN and sodium hydroxide was used, with similar ionic strength and pH as the optimized ammonium hydroxide elution plug. The sodium hydroxide solution ensured elution of G2-PA from the trap column but at the same time impeded ITP, which resulted in very broad G2-PA and free PA zones (Figure S2). This result indicates that the sharp zone is not created by pH differences in the discontinuous BGE, but by ITP with ammonium ions as LE, although an additional pH-induced stacking effect for G2-PA cannot be excluded completely.

An overview of the optimized injection and elution procedure is shown in Figure 2. This procedure was used to characterize the trap-column and analyze additional glycan standards with CZE–DTIMS–MS.

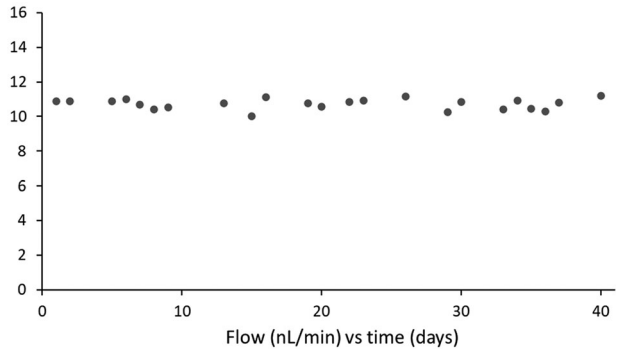


**FIGURE 3** Flow through the capillary electrophoresis (CE) capillary with trapping column, determined by flushing glucose in water through the capillary at a pressure of 1 bar and determining the arrival time at the capillary exit. The capillary with trap column used an inlet capillary length of 10 cm (50 µm id) and an outlet capillary length of 100 cm (50 µm id).

### 3.3 | Characterization of trapping columns

A number of eight trap columns, with MCX and diatomite blends, were successfully prepared on different days with freshly prepared sorbent blends. All columns could be used for at least 100 and up to 340 sample runs, without significantly affecting the flow rate through the columns. The flow rate at 100 mbar, using 50 mM FA, was 10.27 ± 0.03 times lower than at 1 bar ($n = 6$, measured over 67 days), showing a linear response between flow rate and pressure. At a pressure of 1 bar, a flow rate of 10.7 ± 0.3 nl/s was obtained (Figure 3; $n = 23$, measured over 40 days), using an outlet capillary of 100 cm, which demonstrates the consistent flow properties through the trap column that is needed for reproducible injection procedures and robust analyses.

To determine if the sample capacity of the trap column was sufficient for high-volume injections, different volumes of maltoheptaose (G7)-PA were injected onto the column. The injection volume was gradually increased with decreasing G7-PA concentration to obtain equal absolute amounts of analyte on the trap column for all injections. Injection volumes of 1.7–13.6 µl, corresponding to 1.7 pmol amounts on column, provided similar signal intensities for equal injected absolute amounts, demonstrating the high sample capacity of the trap column, with a breakthrough volume >13.6 µl (Figure S3). The largest injection volume is approximately a 1000-fold higher than normally used in CZE, and the improved injection capacity allowed for the CE–IMS–MS analysis of G2-PA with a limit of detection (LOD) of 35 nM, using a 10 µl injection volume. With a 2% injection volume (15.6 nl) on a 70 cm capillary without trapping column and CZE with a 2 M HAc BGE, an LOD of 18.5 µM was obtained for G2-PA. The absolute amount injected for the LOD determination on the trap column system is approximately 80% of the absolute amount injected in the system without trap column, which demonstrates the high retaining capacity of the trap column.

To demonstrate the applicability of in-line trapping columns with ion exchange/diatomite blends for CZE–IMS–MS, the developed trapping method was applied to the CZE–IMS–MS analysis of *N*-glycans. The glycans were derivatized with PA, and a 5 µl sample volume was injected. Sialylated *N*-glycan standards, which are negatively charged at pH values above 2.9, showed a reduced retention on the strong cation exchange material, and therefore, a mixed weak anion and strong cation exchange trap column with diatomite (1:1:2 m/m/m) were prepared to also trap acidic glycans. The weak anion exchange material (DVB–NVP modified with piperazine substituents) can be used to trap and elute anions under the same conditions as optimized for the strong cation exchange sorbent material. Figure 4 shows the CZE–DTIMS–MS analysis of chemoenzymatically synthesized and PA-labeled *N*-glycan standards, using strong cation/weak anion exchange sample trapping. Both neutral and sialylated *N*-glycans, derivatized with PA, were enriched simultaneously without the need for sialic acid derivatization and allowed an analysis of all compounds in positive IMS–MS mode (Figure 4B–H). Ion mobility separation improved selectivity and provided CCS values for identification (Figure 4I–O). Furthermore, the ATDs show broad profiles and partly separated peaks, demonstrating that the *N*-glycans adopt different specific conformations, which can be partially separated by DTIMS and used for the identification of the glycans in complex samples by using their conformer distribution fingerprints and CCS values [8].

## 4 | CONCLUDING REMARKS

SPE sorbent blends with diatomite provided fast and easy filling of trap columns for sample trapping in CE–IMS–MS. The columns were used for up to 340 runs and remained highly permeable with a consistent flow rate during their lifetime. The trap columns were applied to the analysis of acidic and neutral glycans, derivatized with a positive chargeable tag, which were enriched simultaneously by using mixed anion/cation exchange and diatomite sorbents. Sample volumes up to 13.6 µl were injected, which is a factor 1000 higher than with conventional injection methods in CZE, leading to low nM limits of detection for *N*-glycans in positive IMS–MS mode. The described robust sample trapping can be applied broadly with different types of SPE sorbents for both anionic as cationic analytes and is expected to allow large-volume injections

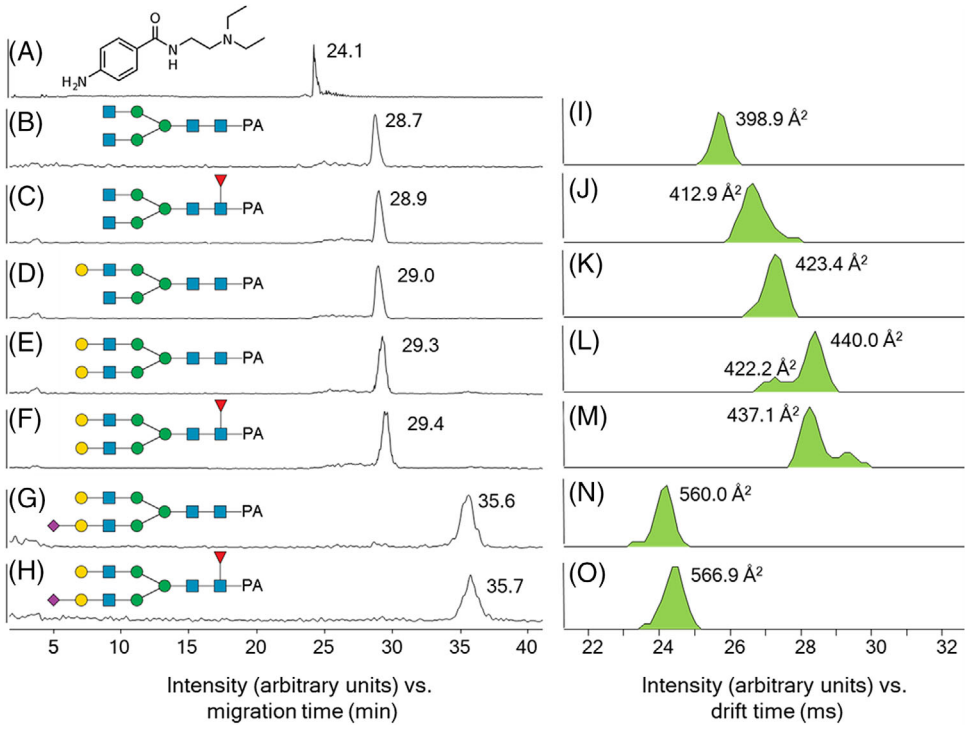


**FIGURE 4** Structures and extracted ion electropherograms of PA (A as $[M+H]^+$ ions) and procainamide (PA)-derivatized *N*-glycans (B–F as $[M+2H]^{2+}$ and G,H as $[M+3H]^{3+}$ ions), obtained during capillary zone electrophoresis (CZE)–ion mobility spectrometry (IMS)–mass spectrometry (MS). Corresponding IMS arrival time distributions and collision cross section (CCS) values of the *N*-glycans are shown in I–O. A 5 µl volume of 5 µM sialylated and 500 nM nonsialylated *N*-glycans was injected onto a mixed mode anion–cation exchange trap column and glycans were analyzed on an 87 cm fluorocarbon (FC)-coated capillary at 30 kV with an assisted pressure of 25 mbar.

with trap columns in CZE–MS to be used with different compound classes.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

## DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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