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Application of electrophoresis in antiretroviral drug analysis: A comprehensive review

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Abstract

Human immunodeficiency virus (HIV) infection continues to pose a substantial global public health challenge, with recent data estimating that around 39 million people are currently living with the virus worldwide. While sub-Saharan Africa carries the highest prevalence, HIV remains a pervasive issue across all regions. HIV-1 affects individuals of all ages, with young adults and marginalized communities often experiencing elevated infection rates. This review provides a comprehensive examination of capillary electrophoresis (CE) techniques utilized for the analysis of HIV-1 antiretroviral drugs in various samples and matrices, drawing on literature from reputable databases spanning the period from 1995 to the present. The reported methods were critically analyzed, compared, and evaluated for their practical applicability in pharmaceutical and clinical settings. The findings highlight that although CE offers notable advantages such as versatility, high efficiency, and cost-effectiveness, its application in the analysis of antiretroviral drugs remains relatively underexplored in this vital therapeutic domain.

Keywords: Capillary electrophoresis, Antiretroviral drugs, Determination

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1. Introduction

Human Immunodeficiency Virus Type 1 (HIV-1) is the primary causative agent of Acquired Immunodeficiency Syndrome (AIDS). It is a retrovirus belonging to the genus Lentivirus within the family *Retroviridae*. HIV-1 primarily targets CD4+ T lymphocytes, weakening the immune system over time. The virus contains single-stranded RNA and uses the enzyme reverse transcriptase to convert its RNA into DNA, which is then integrated into the host's genome, enabling persistent infection (1-4).

HIV-1 remains a major global public health concern. According to the World Health Organization (WHO) and UNAIDS, approximately 39 million people worldwide were living with HIV in recent estimates. The highest prevalence is observed in sub-Saharan Africa, though the virus affects populations globally. HIV-1 impacts all age groups, with young adults and marginalized communities often experiencing higher infection rates (5). Transmission occurs through unprotected sex, sharing contaminated needles, receiving infected blood transfusions, or from mother to child during childbirth or breastfeeding.

The treatment of HIV-1 relies on antiretroviral therapy (ART), which consists of various drug classes that target different stages of the virus's life cycle. These include nucleo-

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side reverse transcriptase inhibitors (NRTIs), which block the reverse transcriptase enzyme to prevent the conversion of viral RNA into DNA; non-nucleoside reverse transcriptase inhibitors (NNRTIs), which bind directly to reverse transcriptase and inhibit its function; protease inhibitors (PIs), which prevent the virus from maturing by blocking the protease enzyme; integrase strand transfer inhibitors (INSTIs), which stop the virus from integrating its DNA into the host cell's genome; entry inhibitors, which prevent the virus from entering CD4 cells; and post-attachment inhibitors, which block the virus from completing the entry process after binding to the cell (6).

A typical treatment plan for HIV-1 involves a combination of drugs from at least two different classes, known as combination antiretroviral therapy (cART). This approach is designed to reduce the viral load to undetectable levels, prevent the progression of the disease, and reduce the risk of transmission. Initiation of ART is recommended for all individuals diagnosed with HIV, regardless of their CD4 count, to improve long-term health outcomes. Regular monitoring of viral load and CD4 count is crucial to assess treatment efficacy and detect potential drug resistance. Additionally, adherence to the prescribed regimen is essential to prevent the development of drug-resistant strains of the virus (7).

High-Performance Liquid Chromatography (HPLC) remains a cornerstone analytical technique across various chemical fields, valued for its versatility, sensitivity, and broad range of applications. HPLC's robust separation capabilities and ability to handle complex matrices make it indispensable in pharmaceutical, environmental, and food analysis.

Capillary electrophoresis (CE), particularly in the form of capillary zone electrophoresis (CZE), offers several advantages including high separation efficiency, low reagent and solvent use, and alignment with green chemistry principles. Its low operational cost and rapid analysis time make it attractive for laboratories with limited budgets. CE is especially effective for charged analytes and has proven useful in pharmaceutical quality control. However, when compared to high-performance liquid chromatography (HPLC), CE has notable limitations.

HPLC generally provides higher sensitivity, better reproducibility, and greater robustness, especially in handling complex or matrix-rich samples like biological fluids. It also supports a broader range of analytes and detectors, making it more versatile for routine and regulated analysis. While CE is environmentally friendly and cost-effective, it can be more sensitive to matrix effects, has lower sample capacity, and requires more expertise for method development. Overall, CE is ideal for specialized or resource-conscious applications, while HPLC remains the preferred choice for high-throughput, high-sensitivity, and broad-scope analyses (9).

However, despite these advantages, CE faces several critical limitations when compared to high-performance liquid chromatography (HPLC). Its lower sensitivity, especially with conventional UV detection, can hinder the analysis of trace-level compounds. Reproducibility of migration times can be compromised by small fluctuations in experimental conditions, affecting quantification accuracy. The technique also struggles with low sample loading capacity, limited detector compatibility, and a narrower range of analytes; particularly neutral or hydrophobic compounds. Method development in CE is often complex, requiring fine control of multiple parameters, and the fragile nature of capillaries adds to operational challenges. Moreover, CE is particularly susceptible to matrix effects in complex samples such as biological fluids, where proteins and salts can interfere with separation performance. In contrast, HPLC offers greater robustness, broader applicability, and superior performance for routine, high-throughput, and matrix-rich analytical tasks (9, 10).

Overall, CE is ideal for specialized or resource-conscious applications, while HPLC remains the preferred choice for high-throughput, high-sensitivity, and broad-scope analyses (9).

Rather than viewing CE and Liquid Chromatography (LC) as competing techniques, they should be regarded as complementary analytical tools. Since CE and LC employ distinct separation principles, they can provide different and valuable analytical

information. Combining both techniques often leads to a more comprehensive understanding of complex samples, enhancing the depth and reliability of analytical results.

CE encompasses a range of separation techniques, each based on distinct separation principles. CZE separates analytes based on differences in their electrophoretic mobilities. Micellar electrokinetic capillary chromatography (MEKC) enables the separation of neutral and charged compounds using surfactant micelles as a pseudo-stationary phase. Capillary gel electrophoresis (CGE) separates analytes by size as they migrate through a gel matrix, which acts as a molecular sieve. Capillary isoelectric focusing (CIEF) focuses and separates zwitterionic analytes according to their isoelectric points within a pH gradient. Capillary electrochromatography (CEC) combines electrophoretic and chromatographic principles, separating analytes within a capillary packed with a stationary phase. This diversity of CE techniques provides versatile and powerful tools for the separation and analysis of a wide range of analytes (10, 11).

CE encompasses a variety of techniques, each offering valuable applications in pharmaceutical analysis. The selection of an appropriate technique is influenced by factors such as sample complexity, component characteristics, analyte properties, and the specific analytical objective. With distinct advantages, each CE method facilitates effective separation and detection of various pharmaceutical compounds.

Anti-HIV drugs are the recent developments of drugs and there is a great need to review the analytical work reported so far in the literatures. Efforts have been made to collect the literature from 1995 up to the present.

There is a significant need to review analytical work on HIV-1 drugs using CE due to the crucial role these medications play in managing the global HIV/AIDS epidemic. Reviewing existing CE methods for HIV-1 drug analysis is essential to assess their development, validation, and reliability, ensuring they provide accurate, sensitive, and reproducible results. Additionally, such a review can highlight advancements and trends in CE techniques, such as MEKC and CIEF, which contribute to better drug characterization. Comparative analysis of different CE approaches may also reveal opportunities for method optimization, leading to improved separation efficiency, reduced analysis time, and lower detection limits. Furthermore, as CE is widely used in pharmaceutical quality control, reviewing its applications in HIV-1 drug analysis ensures compliance with regulatory standards. CE techniques also play a vital role in stability and pharmacokinetic studies, providing valuable insights into drug degradation products and pharmacokinetic behavior, both of which are essential for maintaining treatment efficacy. Importantly, CE aligns with the principles of green analytical chemistry, as it requires minimal solvent and reagent use, making it an eco-friendly alternative to conventional methods. Overall, a comprehensive review of CE applications in HIV-1 drug analysis can bridge knowledge gaps, drive methodological improvements, and support the development of more effective therapeutic strategies.

This review gathers literature from reputable databases spanning from 1995 to the present, examining various CE techniques employed for the analysis of HIV-1 drugs across different samples and matrices. The review discusses the advancements and applications of these techniques in detail, highlighting their relevance and effectiveness in the analysis of HIV-1 medications.

2. Analysis of bulk materials and purity determination

A variety of CE and MEKC methods have been developed for the analysis of antiretroviral drugs and their related impurities, with each study optimizing parameters for specificity, sensitivity, and resolution depending on the compounds under investigation.

Boonkerd et al. (12) developed an MEKC method for the analysis of zidovudine (AZT) and ten related substances using a borate/phosphate buffer at pH 9 with 0.05 M SDS. The method achieved complete separation and showed limits of detection (LOD) ranging from 1.2 to 3.0 µg/mL depending on

the analyte.

Cahours et al. (13) employed a CZE method using a 50 mM formic acid–ammonia buffer at pH 10 for the separation of AZT, stavudine (d4T), and natural nucleosides. This method was sensitive and adaptable for UV and MS/MS detection, achieving LODs as low as 10 ng/mL for d4T.

In another study, Cahours et al. (14) developed a CZE method for ddA, cytosine, dA, and adenine using a low pH buffer (pH 2.5). The method was more sensitive with MS detection (e.g., LOD for ddA: 2 µg/mL) than UV (100 µg/mL), demonstrating applicability to biological matrices.

Mallampati et al. (15) analyzed didanosine (ddI) and thirteen related impurities using CE. Their optimized method used lithium dodecyl sulfate (LiDS) and sodium tetraborate in the buffer system and achieved good resolution with LODs ranging from 1.0 to 5.0 µg/mL for the impurities.

Carvalho et al. (16) presented an MEKC method for ritonavir (RTV) and three synthetic precursors, using a borate buffer with SDS and 18% acetonitrile. The method demonstrated excellent sensitivity with LODs between 0.21 and 2.04 µg/mL, suitable for quality control applications.

Agrofoglio et al. (17) used CE coupled with ESI-MS to analyze AZT, d4T, 3TC, and ddA, employing both low and high pH buffers for improved separation. The method was highly sensitive, capable of detecting analytes at ppb levels (e.g., AZT at 2.5 ppb).

Tuan et al. (18) developed a CE method for the simultaneous analysis of 15 HIV drugs. The system used a complex buffer containing phosphoric acid, surfactants, and organic solvents. Detection at 185 nm allowed LODs between 0.1 and 5 ppm, making it suitable for therapeutic drug monitoring.

Bastos et al. (19) proposed an MEKC method for nelfinavir (NFV) and its impurities using a tetraborate buffer with SDS and methanol. Their method was robust and suitable for impurity profiling, with LODs ranging from 8.03 to 18.7 µg/mL.

Chiral separation was also addressed in studies like that by Krait et al. (20), who used CE with QA-β-cyclodextrin as a chiral selector to analyze tenofovir enantiomers. The method had a low LOD of 0.45 µg/mL and utilized a quality by design (QbD) approach for optimization.

Similarly, Leonard et al. (21) developed a CE method employing a microemulsion system and a diaza crown ether derivative for the enantioseparation of darunavir. Although specific LODs were not reported, the method was effective in separating the enantiomers using UV detection at 265 nm.

Collectively, these studies demonstrate the versatility and adaptability of CE and MEKC techniques for the qualitative and quantitative analysis of antiretroviral agents, with various configurations tailored to specific analytical goals such as impurity profiling, chiral resolution, and trace-level quantification.

A comprehensive overview of the methodologies employed for the analysis of various antiretroviral drugs and their impurities, highlighting the techniques, conditions, detection methods, and sensitivity levels achieved in each study is Provided in Table 1.

3. Analysis of antiretroviral drugs in biological matrices

The accurate quantification of antiviral drugs in biological matrices is essential for pharmacokinetic studies, therapeutic monitoring, and preventing viral resistance. Various studies have employed CE and MEKC to develop sensitive and reliable analytical methods for this purpose.

Alnouti et al. (22) developed a CE method to simultaneously determine zidovudine (AZT) and its metabolite AZT-MP in rat plasma, amniotic fluid, and fetal tissues. The method used protein precipitation for plasma and amniotic fluid, and solid-phase extraction

Table 1. Methodologies employed for the analysis of antiretroviral drugs.

Analytes	Technique	Capillary Details	Buffer Conditions	Detection	LOD (µg/mL)	Ref
AZT & 10 related substances	MEKC	75 µm I.D., 375 µm O.D., 57 cm length	Borate/phosphate buffer (pH 9) with 0.05 M SDS	UV at 266 nm	Thymine: 1.2; Deoxythymidine: 2.1; T-chlorothymidine: 3.0	(12)
AZT, d4T, natural nucleosides	CZE	50 µm I.D., lengths: 47 cm & 67 cm; for MS: 95 cm × 50 µm I.D. × 150 µm O.D.	50 mM formic acid–ammonia buffer (pH 10)	UV at 254 nm; MS/MS	UV: AZT: 50 ng/mL; d4T: 10 ng/mL	(13)
ddA, C, dA, A	CZE	50 µm I.D., 70 cm length	10 mM formic acid–ammonia buffer (pH 2.5)	UV at 254 nm; MS/MS	UV: ddA: 100 µg/mL; MS: ddA: 2 µg/mL	(14)
ddI & 13 impurities	CE	50 µm I.D., 40 cm total, 30 cm effective length	110 mM LiDS, 40 mM sodium tetraborate (pH 8.0)	UV at 248 nm	ddI: 2.5; Impurities: 1.0–5.0	(15)
RTV & 3 precursors	MEKC	75 µm I.D., 375 µm O.D., 60 cm total, 50 cm effective length	15 mM sodium tetraborate (pH 9.6), 30 mM SDS, 18% acetonitrile	UV at 195 nm	0.21–2.04	(16)
AZT, d4T, 3TC, ddA	CZE	75 µm I.D., 375 µm O.D., 37 cm total, 30 cm effective length; for MS: 70 cm × 50 µm I.D. × 150 µm O.D.	Formic acid/ammonia buffers at pH 2.5 & 10	UV at 254 nm; CE-ESI-MS	AZT: 2.5 ppb; d4T: 20 ppb; ddA: 2 ppb; 3TC: 5 ppb	(17)
15 HIV drugs	CE	50 µm I.D., 42.5 cm total, 35 cm effective length	9 mM phosphoric acid, 0.001% SPAS, 30% acetonitrile, 5% ethanol, 15 mM SDS (pH 2.5)	UV at 185 nm	0.1–5 ppm	(18)
nelfinavir & impurities	MEKC	50 µm I.D., 48.5 cm total, 40 cm effective length	25 mM sodium tetraborate (pH 9.24), 9 mM SDS, 10% methanol	UV at 200 nm	NFV: 14.7; Impurities: 8.03–18.7	(19)
Tenofovir enantiomers	CE	50 µm I.D., 40/50.2 cm length	100 mM sodium phosphate buffer (pH 6.4) with 45 mg/mL QA-β-CD	UV at 257 nm	0.45	(20)
Darunavir enantiomers	CE	75 µm I.D., 40 cm total, 31.5 cm effective length	Microemulsion buffer with n-octane, butanol, SDS, sodium tetraborate; diaza crown ether derivative, 10% acetonitrile	UV at 265 nm	Not specified	(21)

Abbreviations: AZT: Zidovudine; d4T: Stavudine; ddI: Didanosine; 3TC: Lamivudine

for fetal tissues. Separation was achieved with an SDS-phosphate buffer system, offering a linear range of 0.5–50 µg/mL and a limit of detection (LOD) of 0.4 µg/g.

Capillary electrophoresis was also applied to quantify protease inhibitors such as indinavir, nelfinavir, saquinavir, and ritonavir in patient serum (23). Initial conditions resulted in suboptimal separation of some analytes, which was improved using a formic acid-acetonitrile background electrolyte. The method showed good linearity over 62.5 ng/mL to 10 µg/mL.

Several MEKC methods have also

been developed for analyzing combinations of antiretroviral drugs. One such method allowed for the simultaneous separation of stavudine (d4T), didanosine (ddI), and either saquinavir or efavirenz in serum, using SDS and phosphate-borate buffer at pH 9.0 (24). The method demonstrated good sensitivity with LODs ranging from 0.3 to 0.4 µg/mL.

nother MEKC method successfully separated ternary combinations of AZT, ddI, and either nevirapine or ritonavir in serum under similar conditions (25). This method exhibited high sensitivity, with LODs as low as 0.1 µg/mL for nevirapine.

A more complex MEKC method was developed for the simultaneous quantification of AZT, D4T, 3TC, and NVP in plasma using a polyimide-coated capillary and a modified buffer containing SDS, methanol, and ethanol (26). This method featured excellent sensitivity with LODs in the nanogram per milliliter range.

A CE method for protease inhibitors (indinavir, saquinavir, nelfinavir, amprenavir, and ritonavir) in serum used both aqueous and non-aqueous electrolyte systems. The aqueous method used phosphoric acid and HDB, while the non-aqueous method used a formic acid–ammonium formate–acetonitrile-methanol system. Both systems achieved low detection limits (0.02–0.24 µg/mL) and good linearity. Due to the improved selectivity; with the non-aqueous electrolyte systems it was possible to separate three of the five protease inhibitor compounds within less than 3 minutes (27).

Pereira et al. (28) developed an electrokinetic chromatography method capable of separating a wide array of antiretroviral agents in serum, including NRTIs, NNRTIs, and PIs. The method used SDS, sodium tetraborate, acetonitrile, and ethanol in the running buffer, demonstrating a linear range of 1-20 µg/mL and LODs between 0.3-0.5 µg/mL.

In contrast, Xu et al. (29) applied MEKC to urine samples to quantify lamivudine, stavudine, and didanosine. Using a buffer with [C14MIm]Br and phosphate, and reversed polarity voltage, they achieved broader linear ranges (up to 400 µg/mL for didanosine) and lower LODs (as low as 0.1 µg/mL) compared to Pereira et al.'s method.

A comparative analysis of Pereira et al. (28) and Xu et al. (29) highlights significant methodological differences due to matrix specificity. Pereira's method, optimized for serum, utilized hydrodynamic injection and SDS-based buffer, whereas Xu's method for urine used electrokinetic injection and ionic liquid-based buffer. Xu's approach provided broader quantification ranges and lower LODs, suggesting higher sensitivity and flexibility.

Overall, these studies demonstrate the versatility and robustness of CE and MEKC for analyzing antiretroviral drugs in various biological matrices, with each method tailored to specific analyte combinations and sample types. The CE/MEKC methods for antiretroviral drug analysis in biological matrices are presented in Table 2.

4. Electrophoretic analysis of antiretroviral in pharmaceuticals dosage forms

Several researchers have developed and validated CE and MEKC methods for the quantification and quality control of antiretroviral drugs in pharmaceutical formulations. These studies illustrate the increasing relevance of CE-based techniques in pharmaceutical analysis, offering specificity, sensitivity, and alignment with green chemistry principles.

Filho et al. (30) introduced a CZE method to quantify the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine. Using uncoated fused-silica capillaries with internal diameters of 75 µm and varying lengths (27.0 cm and 31.5 cm), the analysis was performed under an applied voltage of +25 kV with a 10 mmol/L sodium phosphate buffer at pH 2.5. UV detection at 200 nm and diazepam as an internal standard enabled reli-

Table 2. Summary of CE/MEKC methods for antiretroviral drug analysis in biological matrices.

Analytes	Matrix	Capillary Details	Buffer/System	Voltage / Current	Detection λ (nm)	LOD (µg/mL)	Linear Range (µg/mL)	Ref.
AZT, AZT-MP, AZDU	Plasma, amniotic fluid, fetal tissue	75 µm I.D., 50 cm	40 mM SDS in 50 mM phosphate (pH 7.0)	15 kV	–	0.4 (all analytes)	0.5–50 (tissue)	(22)
Indinavir, Nelfinavir, Saquinavir, Ritonavir	Serum	75 µm I.D., 37 cm	150 mM formic acid + 10% ACN (pH ~2.2)	15 kV	–	–	0.0625–10	(23)
d4T, ddI, Saquinavir / Efavirenz	Human serum	650 µm I.D., 30 cm eff.	18 mM SDS in 15 mM phosphate-borate (pH 9.0)	+15 kV / ~30 mA	210	0.3–0.4	0.5–35.3	(24)
AZT, ddI, Nevirapine / Ritonavir	Human serum	50 µm I.D., 30 cm eff.	18 mM SDS in 15 mM phosphate-borate (pH 9.0)	+15 kV / ~30 mA	210	0.1–0.6	0.5–28.8	(25)
AZT, D4T, 3TC, NVP	Plasma	75 µm I.D., 54.3 cm eff.	5 mM tetraborate + 50 mM SDS + 30% MeOH + 5% EtOH	30 kV	230	0.008–0.015	0.028–8.0	(26)
Indinavir, Saquinavir, Nelfinavir, Amprenavir, Ritonavir	Serum	50 µm I.D., 40 cm eff.	(1) Aqueous: 16 mM H_3PO_4 + 0.001% HDB (pH 2.5); (2) Non-aq: 1 mM HCOOH + 25 mM ammonium formate in ACN:MeOH (80:20)	±30 kV / 25–27 µA	195	0.02–0.24	0.2–50	(27)
Indinavir, Ritonavir, Saquinavir, Nelfinavir, d4T, AZT, ddI, NVP, EFV	Serum	75 µm I.D., 40 cm eff.	20 mM SDS + 10 mM tetraborate + 30% ACN + 5% EtOH	20 kV	–	0.3–0.5	1.0–20	(28)
3TC, d4T, ddI	Urine	50 µm I.D., 40 cm eff.	15 mM [C14MIm]Br + 60 mM phosphate (pH 9.0)	-20 kV	254	0.1–0.3	0.3–400	(29)

Abbreviations: AZT: Zidovudine; AZT-MP: Zidovudine monophosphate; AZDU: Azidothymidine diphosphate; d4T: Stavudine; ddI: Didanosine; 3TC: Lamivudine; NVP: Nevirapine; EFV: Efavirenz; SDS: Sodium dodecyl sulfate; ACN: Acetonitrile; HDB: Hexadimethrine bromide; Eff.: Effective length; I.D.: Inner diameter; MeOH: Methanol; EtOH: Ethanol

able quantification within a range of 80.0 to 120.0 µg/mL, with a limit of detection (LOD) of 1.4 µg/mL.

Prado et al. (31) developed another CZE method targeting indinavir sulfate in both commercial and simulated capsule formulations. Separation was achieved using a 75 µm ID fused-silica capillary (27 cm total length), with 20 mmol/L phosphate buffer at pH 2.52 as the background electrolyte and UV detection at 214 nm. With diazepam as the internal standard, the method demonstrated linearity over 20.0–100.0 µg/mL and an LOD of 4.61 µg/mL. Notably, it successfully distinguished indinavir from impurities such as cis-1-amino-2-indanol and lactone derivatives.

Sekar et al. (32) employed MEKC for simultaneous analysis of lamivudine and zi-

dovudine using a 75 µm ID capillary with a background electrolyte of 12.5 mM sodium tetraborate and 15 mM boric acid at pH 10.8, supplemented with 90 mM sodium dodecyl sulfate (SDS) and 5% acetonitrile. P-aminobenzoic acid (PABA) served as the internal standard, with UV detection at 210 nm. The method was linear from 10–80 µg/mL for lamivudine and 10–100 µg/mL for zidovudine, achieving LODs of 2.5 µg/mL and 2.0 µg/mL, respectively.

In a subsequent study, Sekar et al. (33) expanded their MEKC methodology to simultaneously quantify lamivudine (3TC), stavudine (d4T), and nevirapine (NVP). This method used a longer capillary (73.5 cm total, 62 cm effective) and a buffer containing 10 mM sodium tetraborate (pH 9.8), 100 mM SDS, and 15% 2-propanol. Ibuprofen was used as the internal standard, with detection at 200 nm. The method showed excellent linearity for all analytes—20–200 µg/mL for 3TC, 5–50 µg/mL for d4T, and 25–250 µg/mL for NVP—and achieved low LODs (1.8 µg/mL, 2.0 µg/mL, and 1.6 µg/mL, respectively), demonstrating suitability for routine quality control.

Lago et al. (34) focused on a stability-indicating CZE method for tipranavir (TPV). Utilizing a 50 µm ID capillary (40 cm effective length), the method employed 50 mM sodium borate at pH 9.0 with 5% methanol and an applied voltage of +28 kV. Furosemide was used as the internal standard, with detection at 240 nm. It provided a linear range of 20–200 µg/mL and an LOD of 5.8 µg/mL. Forced degradation studies confirmed its capability to assess TPV stability, and HRMS was used to identify two organic impurities.

Guichard et al. (35) devised three CE systems to cover a broad range of antiretroviral agents. The first system used a 50 mM phosphate buffer at pH 2.5, suitable for formulations containing abacavir, emtricitabine, lamivudine, tenofovir, and rilpivirine. The second system, optimized for efavirenz-containing products, employed a 20 mM phosphate buffer with 20 mM SDS at pH 7.0. The third system was tailored for dolutegravir and raltegravir and used 5 mM carboxymethyl-β-cyclodextrin in 50 mM phosphate buffer (pH 2.5, adjusted with Tris). All systems employed a fused-silica capillary (50 µm ID, 50.5 cm total, 42 cm effective) with sample injection at 50 mbar for 6 seconds and separation voltages of 10-30 kV, depending on the system.

A notable observation across these studies is the widespread use of the one-factor-at-a-time (OFAT) approach for method optimization. While OFAT is straightforward, it is less efficient and may miss interactions between experimental variables. In contrast, a limited number of studies, such as those by Bastos et al. (19) and Leonard et al. (28), implemented advanced experimental designs like factorial and central composite designs, enhancing robustness and efficiency. Furthermore, many of these electrophoretic methods comply with green analytical chemistry principles by minimizing organic solvent usage, reducing reagent consumption, and offering environmentally sustainable operation—making them favorable for routine pharmaceutical analysis.

The CE/MEKC methods for antiretroviral drug analysis in pharmaceutical formulations are summarized in Table 3.

5. Advanced techniques and future directions

Recent advancements in capillary electrophoresis (CE) techniques have significantly enhanced the analysis of HIV drugs, particularly through the integration of CE with mass spectrometry (CE-MS) and the development of microchip-CE systems.

CE-MS has emerged as a powerful tool for the sensitive and specific detection of antiretroviral drugs in complex biological matrices. For instance, Agrofoglio et al. (17) utilized CE coupled with electrospray ionization mass spectrometry (ESI-MS) to detect

Table 3. Summary of CE/MEKC methods for antiretroviral drug analysis in pharmaceutical formulations

Matrix	Analytes	Technique	Capillary Dimensions	Buffer Composition & pH	Detection (nm)	LOD (µg/mL)	Linearity Range (µg/mL)	Internal Standard	Ref
Tablets	Nevirapine (NVP)	CZE	75 µm ID; 27.0 & 31.5 cm (19.4 & 23.0 cm effective)	10 mM sodium phosphate, pH 2.5	200	1.4	80–120	Diazepam (50 µg/mL)	(30)
capsules	Indinavir sulfate	CZE	75 µm ID; 27 cm (19.4 cm effective)	20 mM phosphate, pH 2.52	214	4.61	20–100	Diazepam	(31)
Tablets	Lamivudine, Zidovudine	MEKC	75 µm ID; 27 cm (19.4 cm effective)	12.5 mM Na tetraborate, 15 mM boric acid, pH 10.8, 90 mM SDS, 5% ACN	210	2.5 (3TC), 2.0 (AZT)	10–80 (3TC), 10–100 (AZT)	PABA	(32)
Tablets	Lamivudine, Stavudine, Nevirapine	MEKC	75 µm ID; 73.5 cm (62 cm effective)	10 mM sodium tetraborate, pH 9.8, 100 mM SDS, 15% 2-propanol	200	1.8 (3TC), 2.0 (d4T), 1.6 (NVP)	20–200 (3TC), 5–50 (d4T), 25–250 (NVP)	Ibuprofen	(33)
soft capsules	Tipranavir (TPV)	CZE	50 µm ID; 40 cm effective	50 mM sodium borate, pH 9.0 + 5% methanol	240	5.8	20–200	Furosemide	(34)
Tablets	ABC, FTC, 3TC, TDF, RPV	CE	50 µm ID; 50.5 cm (42 cm effective)	50 mM phosphate, pH 2.5	210	–	–	–	(35)
	EFV, DTG, 3TC	CE	Same as above	20 mM phosphate, pH 7.0 + 20 mM SDS	210	–	–	–	
DTG, RAL (+3TC, ABC)	CE	Same as above	5 mM CMB in 50 mM phosphate (pH 2.5, adjusted with 1 M Tris)	210	–	–	–		

Abbreviations: AZT: Zidovudine; AZT-MP: Zidovudine monophosphate; ABC: Abacavir; FTC: Emtricitabine; 3TC: Lamivudine; TDF: Tenofovir; RPV: Rilpivirine, EFV: Efavirenz, DTG: Dolutegravir, RAL: Raltegravir; SDS: sodium dodecylsulphate; CMB: Carboxymethyl-β-cyclodextri

nucleoside reverse transcriptase inhibitors like AZT, d4T, 3TC, and ddA at sub-ppb levels. The coupling of CE with MS enables not only high-resolution separation but also precise molecular identification, making it ideal for pharmacokinetic and metabolomic studies.

Microchip-CE, a miniaturized version of CE, has also been explored for high-throughput and rapid analysis. These platforms reduce sample and reagent consumption while delivering results in minutes, making them suitable for point-of-care applications. Though still in early stages for antiretroviral analysis, microchip-CE holds great potential for routine therapeutic drug monitoring and personalized medicine, especially in low-resource settings where rapid diagnostics are critical.

Future directions include:

• Wider implementation of CE-MS/MS methods for multi-drug quantification in

Table 4. HIV combinations available in practice.

Dual therapy	1	cabotegravir and rilpivirine (CAB/ PRV)	Triple therapy	1 bictegravir, emtricitabine, and tenofovir (BIC / FTC / TAF)
	2	emtricitabine and tenofovir (FTC / TAF)		2 abacavir, lamivudine, and zidovudine (ABC / 3TC / ZDV)
	3	darunavir and cobicistat (DRV / COBI)		3 doravirine, lamivudine, and tenofovir (DOR / 3TC / TDF)
	4	dolutegravir and lamivudine (DTG / 3TC)		4 efavirenz, lamivudine, and tenofovir (EFV / 3TC / TDF)
	5	abacavir and lamivudine (ABC / 3TC)		5 emtricitabine, rilpivirine, and tenofovir (FTC / RPV / TAF)
	6	dolutegravir and rilpivirine (DTG / RPV)		6 efavirenz, emtricitabine, and tenofovir (EFV / FTC / TDF)
	7	atazanavir and cobicistat (ATV / COBI)		7 emtricitabine, dolutegravir and tenofovir (FTC/ RPV/TAF)
	8	lamivudine and tenofovir (3TC / TDF)		8 dolutegravir, lamivudine and tenofovir(DOL/3TC/ TAF)
	9	lamivudine and zidovudine (3TC / ZDV)		9 dolutegravir, lamivudine and abacavir(DOL/3TC/ ABC)
	10	lopinavir and ritonavir (LPV / RTV)		10 tenfovir, lamivudine and nevirapine(TAF/3TC/ NVP)
				11 lamivudine, zidovudine, nevirapine(3TC/ZDV/ NVP)
multi- drug therapy	1	darunavir, cobicistat, emtricitabine, and tenofovir (DRV / COBI / FTC / TAF)		2 elvitegravir, cobicistat, emtricitabine, and tenofovir (EVG / COBI / FTC / TAF)

patient samples.

• Development of integrated lab-on-a-chip systems combining microchip-CE with on-chip sample preparation and detection.

• Application of quality-by-design (QbD) approaches to systematically optimize CE-based methods.

• Greater focus on chiral separations to support enantioselective drug development and quality control.

Overall, advanced CE technologies are set to play a pivotal role in enhancing HIV drug analysis, offering speed, sensitivity, and miniaturization to support global HIV treatment strategies.

6. Conclusion

Despite its numerous advantages, including high efficiency, rapid analysis, and minimal solvent consumption, capillary electrophoresis (CE) has not achieved the widespread adoption anticipated for the analysis of antiretroviral drugs. While CE offers remarkable versatility and cost-effectiveness, its application in this critical therapeutic area remains relatively limited. Many antiretroviral drug combinations currently available on the market have yet to be explored using CE methods, representing a significant gap in analytical research as only the methods presented in this review were reported, for the combinations of HIV drugs currently available in practice (Table 4). Expanding the use of CE for these drug combinations could provide reliable, eco-friendly, and efficient analytical solutions, particularly for routine quality control and therapeutic drug monitoring. Future research efforts should focus on developing and validating CE methods for these unexplored combinations, thereby unlocking the full potential of this powerful separation technique in antiretroviral drug analysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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