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Precision in capillary electrophoresis with respect to quantitative analysis of suramin

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Abstract

Suramin is an important anti-tumor and anti-viral chemotherapeutic agent. We have previously presented a capillary electrophoresis (CE) method for its quantitative analysis, where its quantitation was linear over three orders of magnitude, with good precision (1.8%) and accuracy. The constantly varying electroosmotic properties of the capillary due to various causes such as analyte adsorption to the inner wall, affect the migration times of analytes during consecutive electrophoresis runs. This results in progressive changes in analyte peak areas, causing less desirable or unacceptable CE assay precision. This paper illustrates a strategy to overcome the problem of assay reproducibility by using an internal standard whose migration time is short and close to that of the analyte so that the relative change of migration time is minimized. Assay precisions as good as 0.3% were observed in these experiments. These results are in agreement with the theoretical basis of experimental capillary electrophoresis.

1. Introduction

Peak-area reproducibility is the most important criterion that leads to good precision in a separation-based assay. Usually, in high-performance liquid chromatography (HPLC), where the technique is very well understood and established, precisions of <1% are routinely achieved. Although capillary electrophoresis (CE) is much more efficient than HPLC, some obvious limitations tend to cause CE precisions to exceed 1%. One of these limitations is the sample loading size. CE is an inherent micro-analytical technique, requiring the use of narrow-bore capillaries. The inner diameters of the capillaries are between 25 and 75 μm [1] for optimum resolutions. Thus, total capillary volumes are in the microliter range, and sample loading volumes are usually at nanoliters or sub-nanoliters [1–3], whereas in HPLC, the sample volumes are in the microliter range. Together with the short detection path of 25 to 75 μm, CE peak areas usually have lower signal-to-noise ratios than those of HPLC. Thus, minute variation in sample loading, voltage, temperature, electrolyte, and capillary can cause considerable variation in the peak-area measurements. With the exception of the capillary, these variations can be eliminated with automation and internal standards. During electrophoresis, analyte molecules can adsorb to the capillary inner wall and affect the zeta potential, thereby changing the electroosmosis. This results in changes in migra-

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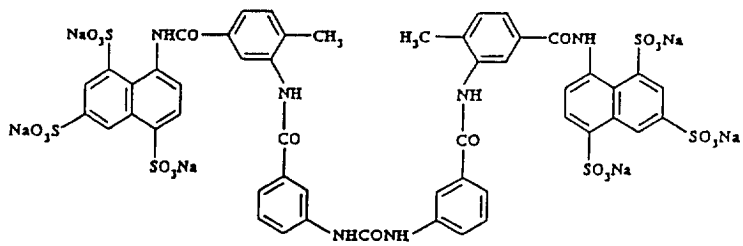
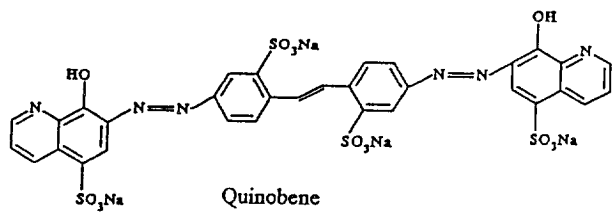
tion time from run to run and affects the reproducibility of the peak areas [4–6]. In order to minimize analyte adsorption, many coating technologies have been developed [7–11]. In our previous presentation on CE assays of quinobene and suramin [6] we discussed the effect of analyte adsorption on electroosmosis. Even with the use of an internal standard, the assay precision was 1.8%. Although this is an acceptable value for CE assays, we believe that it can be further improved.

Suramin, the hexasodium salt of 8,8′-[carbonylbis[imino - 3,1 - phenylenecarbonylimino(4-methyl-3,1-phenylene)carbonylimino]]bis-1,3,5-naphthalenetrisulfonic acid (SU, Fig. 1) is an interesting and useful pharmaceutical. It has a wide range of therapeutic applications [12]. Its original synthesis dates back to 1916 and it was intended for the treatment of parasitic diseases. Later, as the literature reveals, suramin has been widely tested and used, including as an anti-tumor [12,13] and an anti-viral [14–17] drug.

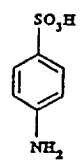
Many HPLC procedures for the analysis of suramin have been reported [18–26]. In the work reported by Klecker and Collins [18] on human plasma and Stolzer et al. [19] on human plasma



Suramin



Quinobene



Sulfanilic acid

Fig. 1. Molecular structures of suramin, quinobene, and sulfanilic acid.

and urine, gradient and isocratic ion-pair HPLC assay methods were described, respectively. These authors have observed good linearities and recoveries in their work. Supko and Malspeis [20], Tong et al. [21], Tjaden et al. [22], and De Bruijn et al. [23] also describe ion-pair HPLC procedures using different types of columns. Fornstedt [24] described peak-distortion effects of suramin due to large system peaks in bioanalysis using ion-pair chromatography. Garcia and Shihabi [25] reported a rapid HPLC procedure for suramin by injecting diluted serum samples directly onto the column.

However, with respect to CE, only two reports appear in the literature on suramin analysis. Garcia and Shihabi [26] reported a rapid CE assay to measure suramin levels in serum using 3-isobutyl-1-methylxanthine as an internal standard. In that work, they have observed a good linearity of peak area versus concentration with a recovery of 93%. However, the precision of the assay was not reported. Hettiarachchi and Cheung [6] reported an internal standard CE assay with a precision of 1.8%. Due to the importance of suramin and the simplicity and separation efficiency of CE, it is beneficial to develop a more precise CE assay that is comparable to HPLC assays.

Our previous study [6] indicated that precision (R.S.D.) of the area of an analyte peak is about 2–3 times that of the migration time, which is inversely proportional to mobility (μ). Mobility of a chemical is a combination of its electrophoretic mobility (μ_{ep}) and the electroosmosis (μ_{eo}) of the capillary, and can be represented by

$$\mu = \mu_{ep} + \mu_{eo} \quad (1)$$

Since μ_{ep} is an inherent property of the chemical, μ will remain constant if μ_{eo} is unchanged during the experiment. Irreversible adsorption of analyte molecules to the capillary wall, which results in μ_{eo} variation ($\Delta\mu_{eo}$), is not expected with the use of capillaries covalently coated with neutral organic polymers. However, experimental data indicated the opposite [6]. The adsorption was probably caused by the hydrophobic interaction of the non-polar part of the analyte with the organic coating. Our efforts to desorb the adsorbed analyte molecules by washing the capillary in between electrophoresis with water and organic solvents were impractical or unsuccessful [27].

For analyte A and internal standard I.S., Eq. 1 can be written as:

$$\mu_A = \mu_{ep,A} + \mu_{eo} \quad (2)$$

$$\mu_{I.S.} = \mu_{ep,I.S.} + \mu_{eo} \quad (3)$$

$$\mu_A/\mu_{I.S.} = (\mu_{ep,A} + \mu_{eo})/(\mu_{ep,I.S.} + \mu_{eo}) \quad (4)$$

Since irreversible adsorption of analytes to the capillary affects only μ_{eo}, Eq. 4 suggests that the ratio $\mu_A/\mu_{I.S.}$ is constant if (i) $\Delta\mu_{eo} \ll \mu_{ep,A}$ and $\mu_{ep,I.S.}$ or (ii) $\mu_{ep,A} \simeq \mu_{ep,I.S.}$. Since $\Delta\mu_{eo}$ is finite, condition (i) can be met if $\mu_{ep,A}$ and $\mu_{ep,I.S.}$ are large. Therefore, by choosing a CE condition and an internal standard that maximizes the mobilities of A and I.S. and minimizes the difference in their mobilities, reproducible peak-area ratio and hence good assay results can be achieved with CE. This paper demonstrates such an approach in the CE assay of suramin (SU), using quinobene, the tetrasodium salt of 4,4′-bis(8-hydroxy-5-sulfo-7-quinoline-azo)-stilbene-2,2′-disulfonic acid (QB) and sulfanilic acid (SA) as potential internal standards (Fig. 1).

2. Experimental

2.1. Reagents

Suramin and quinobene were received from the US National Cancer Institute. Sulfanilic acid was purchased from Eastman Organic Chemicals (Rochester, NY, USA). Sample solutions of 0.1 mg/ml were prepared with distilled water.

Tris(hydroxymethyl)aminomethane (Tris) and boric acid were purchased from Mallinkrodt (Paris, KY, USA). Ethylenediaminetetraacetic acid, disodium salt dihydrate (EDTA) was purchased from Aldrich (Milwaukee, WI, USA).

Polyethylene glycols (PEG) 6K, 12K, 20K, and 35K were from Fluka (Ronokonkoma, NY, USA). These chemicals were of reagent grade.

2.2. Electrolytes

Electrolytes were prepared by dissolving in 0.3 *M* Tris–boric acid–0.002 *M* EDTA (pH 8.6) buffer (TBE) (a) 4% each of 6K, 12K, 20K, and 36K PEG for a total of 16% PEG, (b) 0.5% each of 6K, 12K, 20K, and 36K PEG for a total of 2% PEG, (c) 0.25% each of 6K, 12K, 20K, and 36K PEG for a total of 1% PEG. The TBE buffer was prepared with distilled water.

2.3. Equipment

CE was performed on a BioFocus 3000 electrophoresis system using 36 cm × 50 μm or 24 cm × 50 μm coated fused-silica capillary cartridges. All were purchased from BIO-RAD (Hercules, CA, USA). Electrophoresis was performed with pressure loading, 68.95–413.40 × 10^9 Pa (10–60 p.s.i.) per 1 s, and at 12–18 kV and with UV detection at 254 nm.

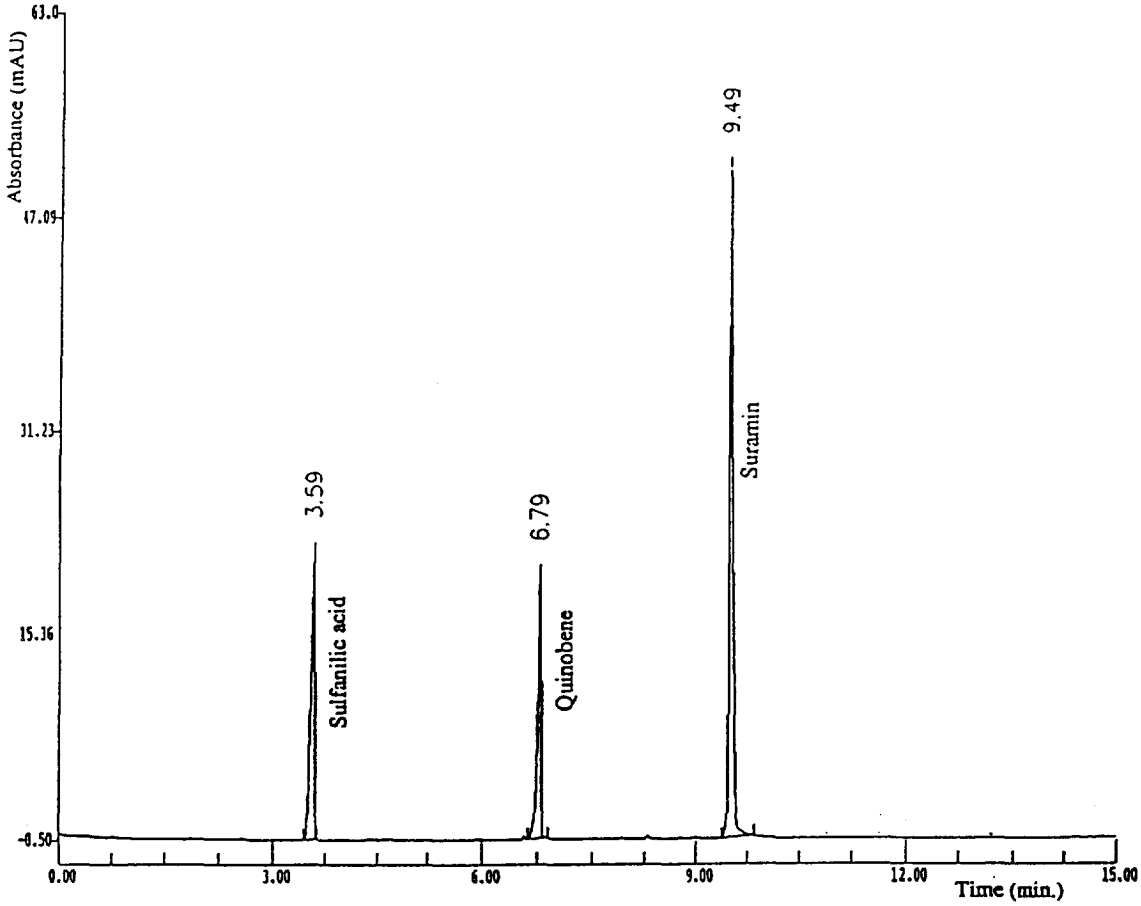


Fig. 2. Electropherogram of a mixture of suramin, quinobene, and sulfanilic acid. Conditions: 16% PEG electrolyte, 24 cm × 50 μm capillary, run voltage 15 kV, pressure injection, 2.67 · 10^9 Pa s (30 p.s.i. s), and detection at 254 nm.

3. Results and discussion

3.1. Reproducibility

To test the hypothesis that precision of an internal-standard CE assay can be improved by increasing mobility μ and decreasing the mobility difference between the analyte and the internal standard, we studied the electrophoresis data from a mixture of sulfanilic acid (SA), quinobene (QB), and suramin (SU). Multiple electrophoreses were performed with 24- and 36-cm coated capillaries and with electrolytes of different viscosity to create different mobilities for the analytes. Fig. 2 is a typical electropherogram of a mixture of SA, QB, and SU.

Consistent with our earlier observation [6], the mobilities of SA, QB, and SU decrease with each use of the capillary. Table 1 presents the mobility and peak-area data from 21 sequential electrophoreses of the mixture. For this experiment, the run voltage was set at 15 kV and loading was by pressure, 50 p.s.i. s. Using a 36-cm capillary and the most viscous electrolyte (16% PEG), the precisions (R.S.D.) of the peak-area ratios for all 21 runs are 2–4%. When they are calculated as seven run blocks, the R.S.D. of the first seven runs improved slightly, to 1–3%. Table 2 summarizes data obtained from a 24-cm capillary and the 16% PEG electrolyte. The run voltage was 15 kV and loading was by pressure at 30 p.s.i. s. In these CE

Table 1
Electrophoretic data from a 36-cm capillary

Run no.	Migration time (min)			Mobility (10^4 cm^2 V^{-1} s^{-1})			Peak-area ratio		
	SA	QB	SU	SA	QB	SU	SU–QB	QB–SA	SU–SA
1	11.62	23.93	33.00	1.08	0.52	0.38	3.42	0.90	3.08
2	11.58	23.85	32.90	1.08	0.53	0.38	3.50	0.93	3.26
3	11.63	23.98	33.12	1.08	0.52	0.38	3.51	0.89	3.13
4	11.70	24.17	33.36	1.07	0.52	0.38	3.52	0.94	3.30
5	11.76	24.31	33.61	1.07	0.52	0.37	3.48	0.91	3.18
6	11.82	24.47	33.85	1.06	0.51	0.37	3.54	0.92	3.26
7	11.97	24.78	34.24	1.05	0.51	0.37	3.42	0.89	3.06
8	12.00	24.91	34.45	1.05	0.50	0.36	3.52	0.93	3.27
9	12.04	25.03	34.66	1.04	0.50	0.36	3.45	0.84	2.89
10	12.08	25.17	34.84	1.04	0.50	0.36	3.52	0.93	3.27
11	12.11	25.27	34.99	1.04	0.50	0.36	3.36	0.91	3.06
12	12.17	25.43	35.26	1.03	0.49	0.36	3.54	0.93	3.29
13	12.37	25.76	35.67	1.02	0.49	0.35	3.50	0.93	3.26
14	12.39	25.86	35.83	1.01	0.49	0.35	3.54	0.94	3.32
15	12.42	25.90	35.90	1.01	0.48	0.35	3.55	0.90	3.21
16	12.42	25.97	36.01	1.01	0.48	0.35	3.57	0.99	3.53
17	12.44	26.04	36.14	1.01	0.48	0.35	3.30	0.98	3.25
18	12.44	26.04	36.16	1.01	0.48	0.35	3.55	0.90	3.19
19	12.47	26.03	36.11	1.01	0.48	0.35	3.40	0.98	3.33
20	12.42	25.96	36.05	1.01	0.48	0.35	3.52	0.92	3.23
21	12.46	26.05	36.19	1.01	0.48	0.35	3.46	0.93	3.92
R.S.D. (%):	All 21 runs			2.6	3.1	3.3	2.0	3.6	3.9
	Run 1–7			1.1	1.3	1.4	1.3	1.8	2.8
	Run 8–14			1.2	1.3	1.4	1.7	3.5	4.6
	Run 15–21			0.2	0.2	0.3	2.7	4.0	3.5

For conditions, see Experimental.

Table 2
Electrophoretic data from a 24-cm capillary

Run no.	Migration time (min)			Mobility (10^4 cm^2 V^{-1} s^{-1})			Peak-area ratio		
	SA	QB	SU	SA	QB	SU	SU–QB	QB–SA	SU–SA
1	3.69	7.06	9.81	1.40	0.73	0.53	1.88	0.73	1.38
2	3.55	6.80	9.52	1.46	0.76	0.54	1.91	0.73	1.39
3	3.50	6.70	9.39	1.48	0.77	0.55	1.91	0.74	1.42
4	3.57	6.80	9.51	1.45	0.76	0.54	1.92	0.74	1.42
5	3.52	6.74	9.46	1.47	0.77	0.55	1.93	0.74	1.44
6	3.50	6.72	9.44	1.48	0.77	0.55	1.90	0.75	1.42
7	3.50	6.69	9.40	1.48	0.77	0.55	1.91	0.76	1.44
R.S.D. (%):	All 7 runs			1.8	1.8	1.4	0.7	1.2	1.6

For conditions, see Experimental.

systems, migration of the analytes, from negative to positive electrode, ran counter to electroosmosis μ_{eo}. Mobility data in Table 2 are about 30–40% faster than those in Table 1, due to reduction in μ_{eo} in the shorter capillary. The R.S.D.s of the peak-area ratios from the 24-cm capillary were improved to 0.7–1.6%, about half of the result observed for the 36-cm capillary.

Improvements in the peak-area ratio precision are predictable and reproducible. Table 3 shows the summary of electrophoretic data from experiments performed on separate days and with 24-cm capillaries. Three electrolytes containing 16%, 2%, and 1% PEG in TBE buffer were used to create differences in mobility. As the viscosity of the electrolyte decreased from 16% to 1% PEG, an increase in the mobilities of the chemicals was observed. This is consistent with Stoke's law that electrophoretic mobility is inversely proportional to the viscosity of the electrolyte.

While by increasing the mobility of the analyte and internal standard the improvement of the precision of the CE assay has been demonstrated, decreasing the mobility difference between the analyte and the internal standard is equally effective in improving assay precision. Examine the peak-area ratio R.S.D. data versus normalized delta mobility, which is defined as the difference in mobility of the pair over its average in Table 3. The peak-area ratio R.S.D. of the SU–QB pair, which has the least normalized delta mobility of 0.3, is significantly better than those of the other two pairs, even though SU and QB are the slower migrating ones. The normalized delta mobilities of the other two pairs, QB–SA and SU–SA, were respectively 0.6 and 0.9. Table 3 confirms that the R.S.D. of the peak-area ratio decreases with the normalized delta mobility. Thus, by choosing an internal standard (QB) which gives a small normalized delta mobility with the analyte, a CE assay of 0.6% R.S.D. can be routinely achieved for suramin.

3.2. Specificity

Using a 24-cm capillary, separation of suramin (SU) from the chosen internal standard (QB) was comparable for all three electrolytes discussed earlier (Fig. 3). When the 1% PEG electrolyte was used, the I.S. migrated closely with a significant decomposition product of SU (Fig. 4). While both 16% and 2% PEG electrolytes gave good separation for the I.S. and its decomposition products, the 2% PEG gave larger mobility for the analytes than the 16% PEG electrolyte and thus was chosen for the assay.

Table 3
Precision and "ruggedness" of peak-area ratio in an internal standard CE assay

Experiment no.	Run voltage (kV)	Electrolyte	Migration time (min)			Mobility (10^4 cm^2 V^{-1} s^{-1})			Normalized delta mobility			Peak-area ratio R.S.D.			n
			SA	QB	SU	SA	QB	SU	SU–QB	QB–SA	SU–SA	SU–QB	QB–SA	SU–SA	
1	15	16% PEG	3.55	6.79	9.50	1.46	0.76	0.54	0.33	0.63	0.91	0.72	1.18	1.56	7
2	15	16% PEG	3.58	6.92	9.78	1.44	0.75	0.53	0.34	0.64	0.93	0.84	1.14	1.10	5
3	15	16% PEG	3.68	7.08	9.91	1.41	0.73	0.52	0.33	0.63	0.92	0.52	1.22	1.54	7
4	13	16% PEG	4.28	8.21	11.50	1.39	0.73	0.52	0.33	0.63	0.92	0.62	0.85	1.22	7
5	15	2% PEG	3.73	5.91	7.80	1.39	0.88	0.66	0.28	0.45	0.71	0.69	1.07	1.56	9
6	12	2% PEG	4.46	7.67	10.23	1.45	0.84	0.63	0.29	0.53	0.79	0.58	1.59	1.99	9
7	15	2% PEG	3.55	6.07	8.11	1.46	0.85	0.64	0.29	0.52	0.78	0.31	0.53	0.65	9
8	18	2% PEG	2.89	4.92	6.58	1.49	0.88	0.66	0.29	0.52	0.78	0.61	0.66	1.13	11
9	18	1% PEG		2.51	3.14		1.72	1.37	0.22			0.51			6
										Average:		0.60	1.03	1.34	

For conditions, see Experimental.

3.3. Linearity

Table 4 presents the linearity data on the improved CE assay of suramin. The assay used a 24 cm × 50 μm coated capillary, 2% PEG electrolyte, 15 kV, pressure loading at 15 p.s.i. s, and QB (0.25 mg/ml water) as the internal standard. In an analyte solution concentration range of 0.1 μg/ml to 1.0 mg/ml in I.S., the peak area-ratio of SU–QB (y) was linearly proportional to the

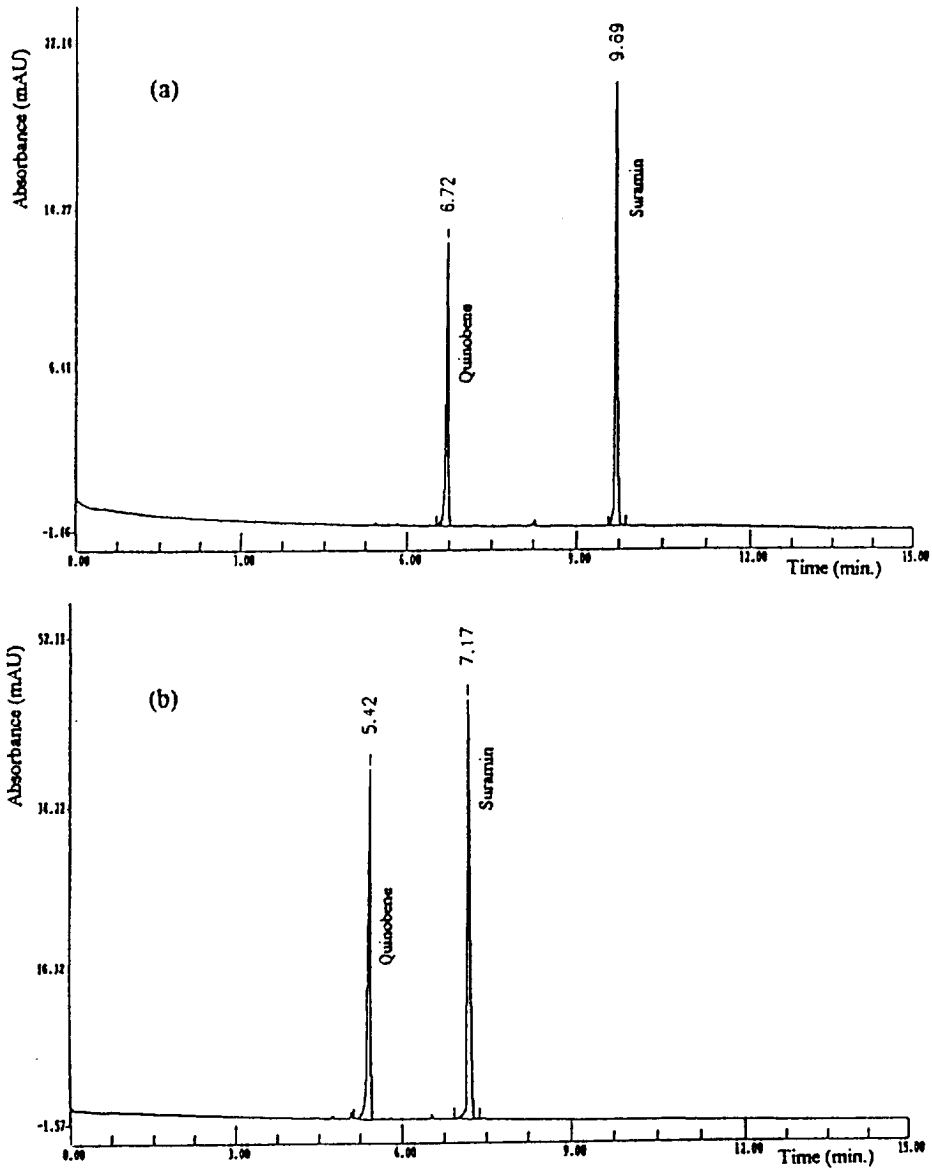


Fig. 3.

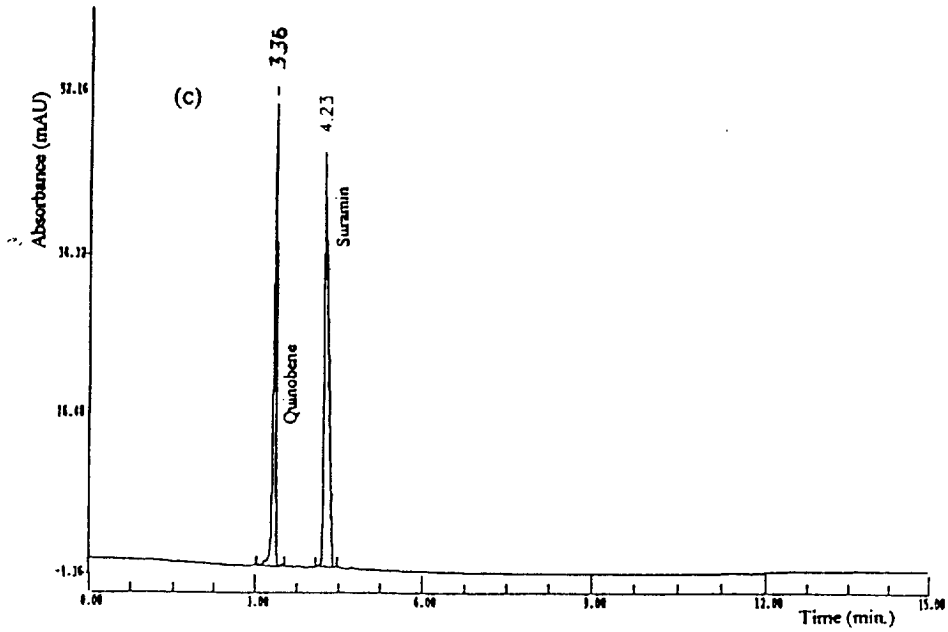


Fig. 3. Electropherograms of suramin and quinobene in (a) 16% PEG electrolyte, (b) 4% PEG electrolyte, and (c) 1% PEG electrolyte; other conditions: 24 cm × 50 μm capillary, run voltage 15 kV, pressure injection, $2.67 \cdot 10^9$ Pa s (30 p.s.i. s), and detection at 254 nm.

Table 4
Linearity data

Run no.	SU (mg/ml I.S.)	Peak-area ratio SU–QB
1	0.00013	0.00726
2	0.00126	0.04279
3	0.01256	0.42698
4	0.12560	4.11397
5	0.22130	7.17635
6	0.29080	9.41390
7	0.45430	14.48543
8	0.48380	15.37550
9	0.61700	19.43455
10	0.68580	21.64488
11	0.88350	27.32580
12	0.94500	29.25927
13	1.09500	33.56189
Slope	30.7913	
Std. error of slope	0.1836	
y-Intercept	0.2450	
Std. error of y est.	0.2403	
r^2	0.9996	

For conditions, see Experimental.

analyte concentration (x) according to the linear equation $y = 30.791x + 0.245$ with an r^2 of 0.9996.

4. Conclusion

Over the past few years, CE techniques have shown a tremendous growth in terms of efficiency, quantitation, and automation. By overcoming the systemic limitations and drawbacks, CE researchers are now achieving much better precisions. When the ease of operation and efficiency are considered, CE stands out in comparison to HPLC. Environmentally speaking, CE generates minute solvent waste in comparison to HPLC. Although many researchers [6,28–36] have shown that quantitation precision approaching that of HPLC was achievable, widespread use of CE assay in pharmaceutical analysis has not appeared yet. Due to a lack of full understanding of the quantitation limitation of a CE assay, many hesitate to use it as a routine

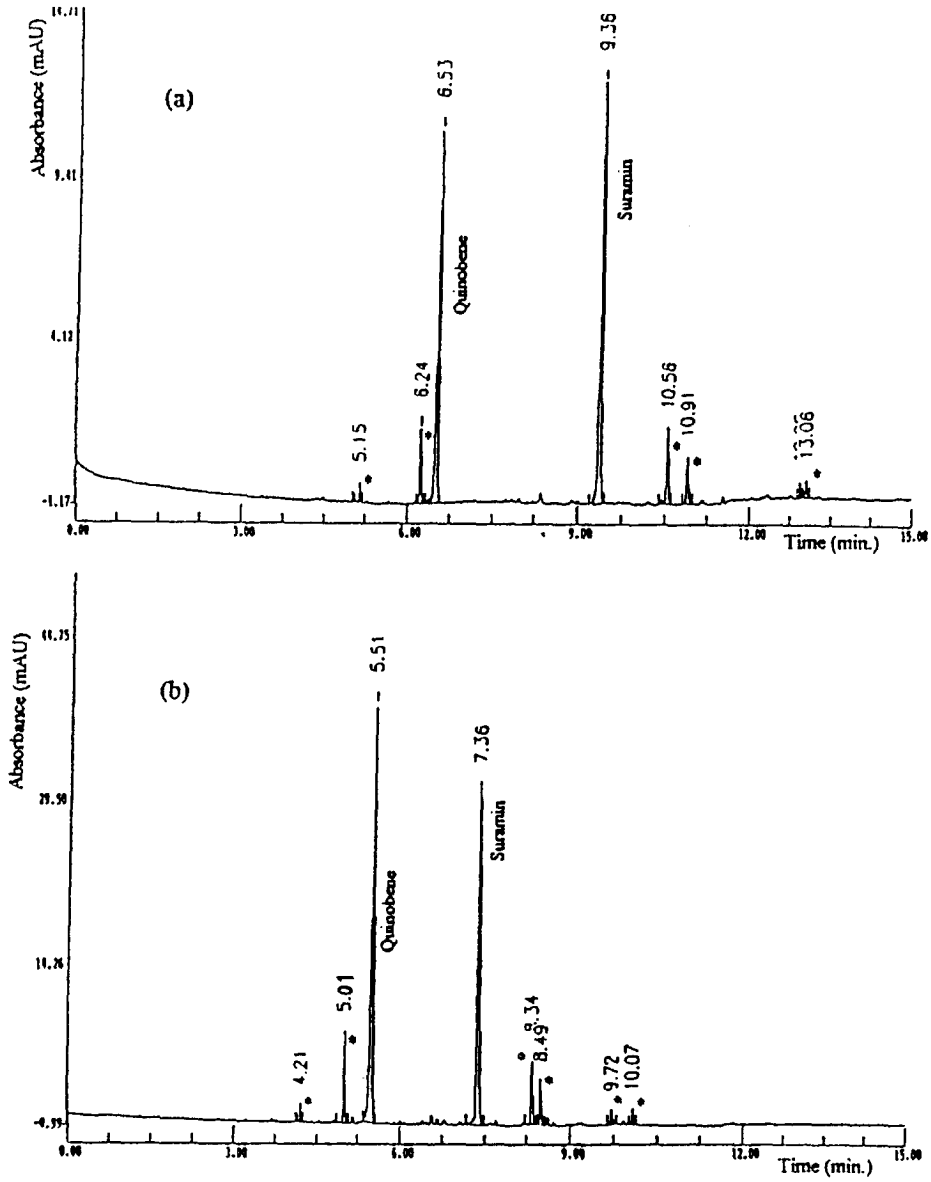


Fig. 4.

quantitative technique. In our previous paper [6] and here, we have discussed the quantitative limitations of CE and strategies to overcome them. In this paper, we demonstrated that by proper choice of an internal standard and the mobility of the analytes, an accurate and rugged CE assay with precision equal to that of HPLC can be routinely achieved. With these and other demonstrations, the authors hope that CE assays will eventually be accepted as a routine assay, complementary to HPLC assay, by the pharmaceutical establishment.

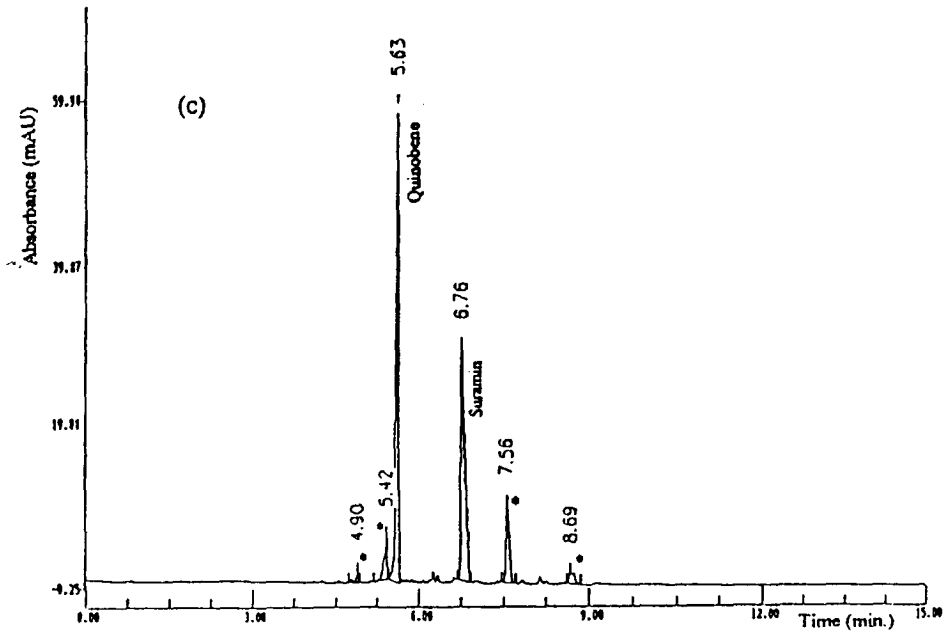


Fig. 4. Electropherograms of a mixture of thermally decomposed suramin and quinobene in (a) 16% PEG electrolyte, (b) 4% PEG electrolyte, and (c) 1% PEG electrolyte; other conditions: 24 cm × 50 μm capillary, run voltage 15 kV, pressure injection, $2.67 \cdot 10^9$ Pa s (30 p.s.i. s), and detection at 254 nm. Asterisks denote decomposition peaks of suramin.

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