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Capillary electrophoretic determination of inorganic ions in a prenatal vitamin formulation

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ABSTRACT

Methods for the quantitative analysis of three cations (calcium, iron and zinc), and the qualitative analysis of several anionic species (chloride, sulfate, nitrate, citrate, fumerate, phosphate, carbonate and acetate) from a prenatal vitamin formulation by two different capillary ion electrophoresis methods are reported. The cation method was evaluated for detection and quantitation limits, precision, accuracy and linearity. For standard solutions, detection limits into the ng/ml range, and reproducibility that averaged less than 1% for migration time and 2% for area response were generated. Calibration plots exhibited linearity in excess of three orders of magnitude. In addition, excellent agreement between capillary ion electrophoresis and flame photometry quantitative results for the cation analyses were obtained.

INTRODUCTION

The use of capillary electrophoresis, especially micellar electrokinetic capillary chromatography (MECC), for the analysis of small organic molecules is quickly becoming a valuable analytical tool in the pharmaceutical laboratory [1]. This is due to the fact that these types of compounds, present as active ingredients, often constitute the major proportion of the formulation. Of increasing importance, however, are small-molecular-mass ionic species frequently present in the same formulation. This paper describes the use of an emerging capillary electrophoretic technique called capillary ion electrophoresis (CIE; Waters' trade name Capillary Ion Analysis, CIA) that utilizes indirect ultraviolet (UV) detection to simplify the analysis of small molecular weight ionic species. Reports of the application of electrophoresis to the determination of these types of ions dates back to at least 1967 [2]. More recently, however, reports have appeared in the literature refining the methods and techniques involved for both anionic [3] and cationic [4,5] species. We have adapted the use of these methods for the analysis of these species in a prenatal vitamin formulation. Such species are currently analyzed by ion chromatography (IC), flame photometry (ICP) or atomic absorption (AA). CIE offers several potential advantages including speed, cost, sensitivity and selectivity, as well as offering an alternate to the established methods for the confirmation of results.

EXPERIMENTAL

Apparatus

A Waters Quanta 4000 capillary electrophoresis system was used throughout (Millipore Waters Chromatography, Marlboro, MA, USA). All separations were performed on standard untreated capillaries, 60 cm × 75 μm I.D. Anion analysis was performed using a negative power supply, with indirect UV detection at 254 nm. Cation analyses were performed using a positive power supply, with indirect UV detection at 185 nm. Data were collected and processed on an 845 Chromatographic Data Workstation (Millipore) at 10 points/s. Injections were performed hydrostatically for 30 s (10 cm height). An applied voltage of 20 000 V was used throughout.

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Chemicals and supplies

All chemicals were purchased commercially from either Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA) in the highest purity available, and were used as is without further purification.

Electrolyte solutions

Prepackaged electrolyte solutions for CIE are commercially available and were obtained from Millipore, prepared in Milli-Q water (Millipore), and used as directed without modification. The anion electrolyte was 5 m*M* chromate, 0.4 m*M* CIA-Pak OFM anion BHT, pH 8.0. The electrolyte used for the analysis of cations was 5 m*M* UV-CAT-1, 6.5 m*M* 2-hydroxyisobutyric acid, pH 4.4.

Detection limits and reproducibility

Detection limits were defined as a signal-to-noise ratio (S/N) of 3. Quantitation limits were defined as a S/N of 10. S/N was determined at 1.0 μg/ml, and extrapolated to the appropriate limit. Reproducibility was determined at 1.0 μg/ml, chosen to closely approximate the quantitation limit. All data is an average of three determinations.

Sample preparation and quantitation

For quantitative analysis three tablets of a common commercially available over the counter prenatal vitamin formulation were assayed. Sample preparation consisted of adding a tablet to 500 ml of water adjusted to pH 2.0 with 6 *M* nitric acid. The solution was then sonicated for 30 min, followed by a 5-min stirring. Volumes of 10 ml of the resulting solution were filtered using a Millex HA filter (Millipore), discarding the first ml. The filtrate was then diluted 1:50 with water and injected. Stock standards were prepared in water at the 1000 μg/ml level and diluted to the desired concentration. Quantitation was performed by single point external standard calibration. An aliquot of each sample analyzed by CIE was sent to the laboratory of the Chemistry Department of the University of Vermont (Burlington, UT, USA) for the ICP analyses.

RESULTS AND DISCUSSION

Method validation

According to the United States Pharmacopeia (USP) [6] there are five parameters that need to be adequately investigated and documented to validate a method. These parameters are precision, accuracy, detection and quantitation limits, linearity and selectivity. With the exception of selectivity, which has been adequately documented previously [4,5], each of these parameters was investigated in turn. Linearity was evaluated from 100 μg/ml to the detection limits for a representative sample of inorganic anionic and cationic species. Acceptable linearity of peak area response was obtained over the entire concentration range with correlation coefficients ranging from 0.998 to 1.000. Detection (S/N = 3) and quantitation (S/N = 10) limits are shown in Table I. Excellent sensitivity with detection limits to the ng/ml level and quantitation limits to the 1.0 μg/ml level are indicated. The precision of peak area response and migration time were evaluated by per-

TABLE I

CIE DETECTION LIMITS, QUANTITATION LIMITS AND REPRODUCIBILITY

Conditions as outlined in Experimental. Data are for ten replicate injections. For reproducibility a 1.0-μg/ml standard solution of each species was evaluated to represent an approximation to the quantitation limit.

Species	Detection limit, S/N = 3 (ng/ml)	Quantitation limit, S/N = 10 (μg/ml)	Migration time, R.S.D. (%)	Area, R.S.D. (%)
Calcium	274	0.913	0.82	2.23
Zinc	313	1.04	0.84	2.06
Iron	326	1.09	0.86	1.77
Sulfate	157	0.523	0.17	1.90
Nitrate	210	0.700	0.17	1.58

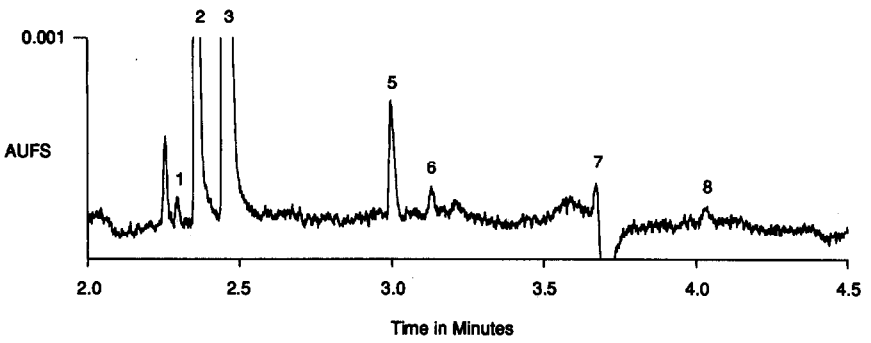

Fig. 1. Electropherogram of prenatal vitamin tablet extract using anion method. Electrolyte: 5 m*M* chromate, 0.4 m*M* CIA-Pak OFM anion BHT, pH 8.0. An untreated capillary, 60 cm × 75 μm I.D., a negative power supply, with indirect UV detection at 254 nm was used. Injections were performed hydrostatically for 30 s (10 cm height). An applied voltage of 20 000 V was used throughout. Peaks: 1 = chloride; 2 = sulfate; 3 = nitrate; 4 = citrate; 5 = fumerate; 6 = phosphate; 7 = carbonate impurity; 8 = acetate. Unlabeled peaks are unknowns.

forming 10 replicate injections, as summarized in Table I. A 1.0-μg/ml standard solution of each species was chosen for this evaluation to represent an approximation to the quantitation limit (Table I).

Prenatal vitamin tablet quantitation

Following sample preparation the vitamin tablet extract was analyzed for both anionic and cationic content. The result of the anion analysis is shown in

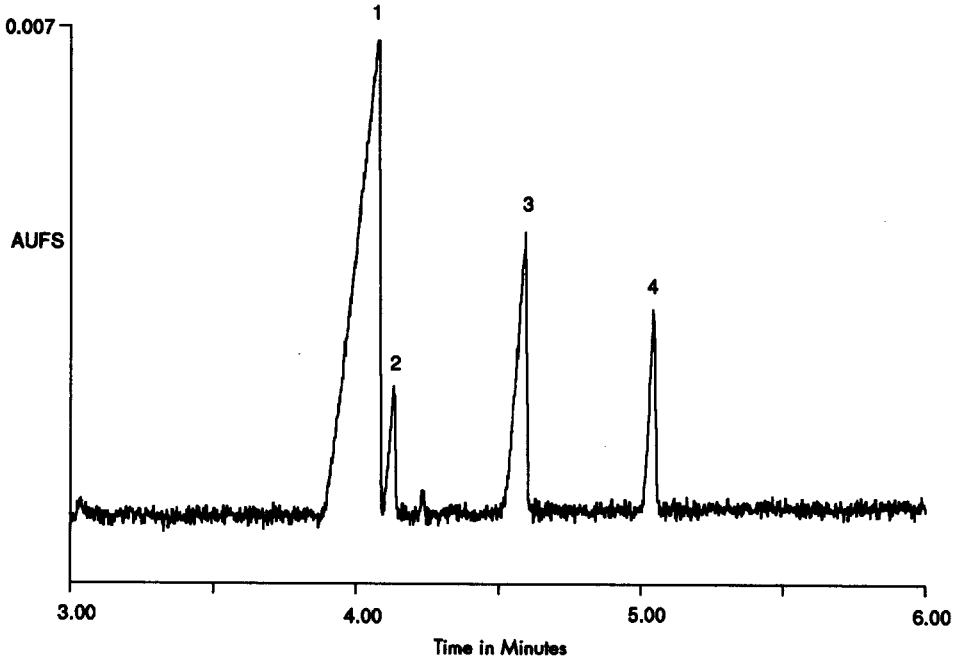

Fig. 2. Electropherogram of prenatal vitamin tablet extract using cation method. Electrolyte: 5 m*M* UV-CAT-1, 6.5 m*M* 2-hydroxyisobutyric acid, pH 4.4. An untreated capillary, 60 cm × 75 μm I.D. and a positive power supply with indirect UV detection at 185 nm was used. Peaks: 1 = calcium; 7.2 μg/ml; 2 = sodium, 0.560 μg/ml; 3 = iron(II), 2.31 μg/ml; 4 = zinc, 0.950 μg/ml.

TABLE II

VITAMIN TABLET CATION QUANTITATION BY CIE

Conditions as detailed in Experimental. Data is the average of triplicate determinations in μg/ml.

Species	Tablet 1		Tablet 2		Tablet 3	
	CIE	ICP	CIE	ICP	CIE	ICP
Calcium	7.20	7.84	7.75	8.60	8.04	9.02
Iron	2.31	2.10	2.66	2.42	2.58	2.46
Zinc	0.95	1.07	0.99	1.15	1.03	1.13

Fig. 1. All of the anionic species identified on the label of the formulation were identified. Quantitation of the levels of each of these species was not performed in this case due to the fact that the levels were not reported on the label. Levels of three of the four cationic species present in the formulation were documented on the label, however, and were used to assess the quantitative accuracy of CIE in comparison with ICP. Fig. 2 presents the results of the cationic analysis on the identical sample used to generate Fig. 1, and submitted for ICP analysis. The comparison of the quantitative results between the two analytical methods (CIE and ICP) for the analysis of three separate tablets is summarized in Table II. Given the 2% error of CIE (Table I) and the 2–9% error obtained from the ICP measurements, these numbers can be shown to overlap within the experimental error and are therefore equivalent. It should also be noted that CIE possess the ability to speciate between iron(II), the species present in the formulation, and iron(III), while ICP does not.

CONCLUSIONS

The results of this work indicate that CIE is a viable alternative to the more traditional methods of analysis for samples of this type, as well as offering significant improvements in efficiency and analysis time. As defined by USP criteria, the reproducibility, linearity, selectivity and sensitivity of this technique are adequate to validate the types of assays used in the pharmaceutical industry. Work continues in our laboratory towards the adoption of these methods to other types of samples of interest in this area.

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REFERENCES

1 M. E. Swartz, *J. Liq. Chromatogr.*, 14 (1991) 923.
2 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.
3 P. Jandik, W. R. Jones, A. Weston and P. R. Brown, *LC · GC*, 9 (1991) 634.
4 W. R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
5 M. Koberda, M. Konkowski, P. Youngberg, W. R. Jones and A. Weston, *J. Chromatogr.*, 602 (1992) 235.
6 *United States Pharmacopeia XXII, NF XVII*, United States Pharmacopeia Convention, Rockville, MD, 1990.