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Journal of Chromatography A, 1051 (2004) 291–296
JOURNAL OF CHROMATOGRAPHY A
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Determination of folic acid in tablets by microemulsion electrokinetic chromatography

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Abstract

A microemulsion electrokinetic chromatography (MEEKC) method has been developed and validated for the determination of folic acid, a water-soluble vitamin, in a commercial tablet formulation. The analysis was performed using a microemulsion containing 0.5% (w/w) ethyl acetate, 1.2% (w/w) butan-1-ol, 0.6% (w/w) sodium dodecyl sulfate, 15% (v/v) 2-propanol and 82.7% (w/w) 10 mmol L^{-1} sodium tetraborate aqueous buffer at pH 9.2. Direct UV detection at 214 nm led to an adequate sensitivity without interference from sample excipients. For quantitative purposes, niacin was used as internal standard. Acceptable precision (<1.2% relative standard deviation (R.S.D.)), linearity (r = 0.9992; range from 160.0 to 240.0 μg/mL), sensitivity (limit of detection (LOD) = 2.98 μg/mL; limit of quantification (LOQ) = 9.05 μg/mL) and recovery (99.8 ± 1.8% at three concentration levels) were obtained. Based on the performance characteristics, the proposed methodology was found suitable for the determination of folic acid in tablet formulations.

Keywords: Folic acid; Vitamins; Microemulsion electrokinetic chromatography

1. Introduction

Vitamins are a group of essential compounds for the development and normal growth of living beings. Absence of vitamins causes serious physiological problems. In technologically advanced societies, vitamin deficiency results mainly from poverty, food faddism, misuse of drugs, chronic alcoholism, or prolonged parenteral feeding. The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and therefore, must be supplied in sufficient amounts with diet [1]. If this intake is insufficient or if special dietary requirements exist multivitamin preparations can be taken in order to prevent vitamin deficiency [2].

Folic acid, *N*-[*p*-{[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino}benzoyl]-L-glutamic acid [3,4] (Fig. 1), also known as folate, is Vitamin B found in some enriched foods and vitamin pills. Folate deficiency is believed to be the most common vitamin deficiency in the world due to food processing, food selection, and intestinal disorders. In the body, folic acid may be converted into any of the active forms of folate. Folate acts as a coenzyme in several single carbon transfer reactions to synthesize DNA, RNA and protein components. Folic acid is a hematopoietic vitamin occurring free or combined with one or more additional molecules of L(+)-glutamic acid in liver, kidney, mushrooms, spinach, yeast and green leave grasses [5].

For the analysis of folic acid and folates, high-performance liquid chromatography (HPLC) has been the technique of choice [6–10]. Even though HPLC is considered an established technology in the pharmaceutical scenario with sensitive and specific methods, HPLC has the disadvantage of complicated system operation and maintenance, requiring large sample and solvent volumes, high cost of consumable supplies and the generation of substantial quantities of hazardous organic solvents, resulting in high disposal costs.

Capillary electrophoresis (CE) has emerged as a powerful analytical tool for rapid separation of analytes. This technique overcomes many of the drawbacks of HPLC. CE has

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doi:10.1016/j.chroma.2004.08.042

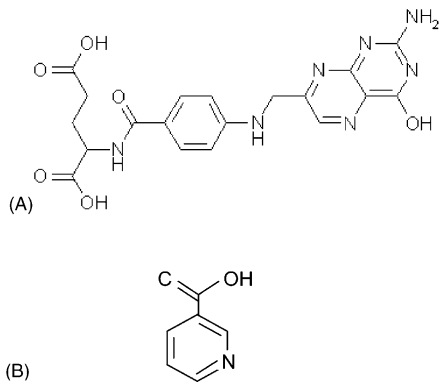

Fig. 1. Chemical structures of folic acid (A) and niacin (B).

advantages of high column efficiency, requiring small sample and solvent volumes with low operating and consumable costs. In nearly all operations of CE, simple aqueous solutions or micelle solutions are used as running buffer. In addition to these usual buffers, microemulsions as separation media for CE has been developed in 1991 by Watarai [11] and the technique has become termed microemulsion electrokinetic chromatography (MEEKC). MEEKC is a relatively new technique which accomplishes electrokinetic separations using buffers containing surfactant coated oil droplets. The technique offers the possibility of highly efficient separation of both charged and neutral solutes covering a wide range of water solubilities [12,13]. The technique separates solutes based on both hydrophobicity and electrophoretic mobility differences. The microemulsion buffers used are composed of minute water-immiscible oil droplets suspended in an aqueous buffer. Typically microemulsions consist of a surfactant (usually sodium dodecyl sulfate (SDS)), an immiscible oil such as octane or heptane, a co-surfactant, butan-1-ol, and an aqueous buffer [14]. Due to the high surface tension between octane and water, a high SDS concentration is required to allow droplet formation. On the other hand, high SDS contents lead to high operating currents limiting the applied voltage to low levels and therefore compromising the overall analysis time. Alternatively, oils with lower surface tension such as di-*n*-butyl tartrate [15] or ethyl acetate [16] might be used allowing microemulsions to be formulated with lower SDS contents (ca. 0.6% (w/w) or lower).

To our knowledge, MEEKC determination of folic acid has not been demonstrated previously. Therefore, the aim of this work was to develop a fast, simple, specific, accurate and precise microemulsion electrokinetic chromatography method for the determination of folic acid in commercial pharmaceutical tablets.

2. Experimental

2.1. Chemicals

All solvents were HPLC grade and were employed as supplied by manufacturer. 2-Propanol was obtained by Merck (Darmstadt, Germany). Sodium dodecyl sulfate was obtained from Riedel-de Haën (Seelze, Germany), ethyl acetate was from Carlo Erba (Sao Paulo, SP, Brazil), butan-1-ol was from Mallinckrodt Baker (Xalostoc, Edo de Mexico, México) and sodium tetraborate-10-hydrate and sodium hydroxide were from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

The experiments were carried out on a Beckman PACE/MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA), equipped with an on-column diode-array detection (DAD) system set at 214 nm and a temperature control device set at 25 °C. A software for data acquisition and treatment (32 Karat^{TM} Software v. 4.0) was used for peak integration and data analysis. Samples were introduced onto the capillary via hydrodynamic injection by applying 0.5 psi for 5 s (1 psi = 6894.76 Pa). The instrument was operated under positive polarity (injection end of the capillary). A constant voltage of +28 kV was used (current of approximately 64 μA) for all experiments. The pH of the buffer was measured with a Digimed DM 21 (Sao Paulo, SP, Brazil) pH meter. An ultrasonic bath Model T-7 Thornton (Sao Paulo, Brazil) was used to degas the electrolyte.

2.3. Capillary conditioning and procedures

Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 75 μm and a total length of 40.2 cm (30 cm effective length) were used. The capillary was thermostated at 25 °C by cooling liquid circulation. New capillaries were conditioned with a 1 mol L^{-1} NaOH solution (30 min), followed by deionized water (20 min) and microemulsion (30 min). At the beginning of the day, the capillary was conditioned with 1 mol L^{-1} NaOH for 15 min, followed by deionized water for 10 min and then microemulsion for 15 min. Between runs, the capillary was rinsed with 1 mol L^{-1} NaOH for 1 min and microemulsion for 2 min. At the end of the day, a final 5 min washing with 1 mol L^{-1} NaOH followed by water was performed.

2.4. Preparation of the microemulsion

Microemulsion was prepared on a w/w basis except for the organic additive (2-propanol (v/v)). The final microemulsion used for separation of the water- and fat-soluble vitamins was prepared by adding 0.5 g ethyl acetate, 1.2 g butan-1-ol, 0.6 g sodium dodecyl sulfate, 15 mL 2-propanol and 82.7 g of sodium tetraborate buffer (10 mmol L^{-1} at pH 9.2) to a 100 mL flask. This mixture was sonicated for 30 min to aid dissolution and an optically transparent and stable microemulsion was obtained.

2.5. Standards and samples

Standards of Vitamins B_{11} (folic acid) and B_3 (nicotinic acid or niacin, used as internal standard), structures on Fig. 1A and B, respectively, Vitamins B_1 (thiaminiummononitrate), B_2 (riboflavine), B_6 (pyridoxol hydrochloride, C (ascorbic acid) and E (α-tocopherol acetate) were all supplied by Merck (Darmstadt, Germany). Samples were commercially available tablets (supplied by laboratory A) containing 5.0 mg of folic acid and excipients in sufficient quantity for a tablet (ca. 145 mg).

2.6. Preparation of standard solutions

Standard stock solutions of folic acid (2000.0 μg/mL), niacin (4000.0 μg/mL), thiaminiummononitrate (2000.0 μg/mL), riboflavine (2000.0 μg/mL), pyridoxol hydrochloride (2000.0 μg/mL), ascorbic acid (4000.0 μg/mL) and α-tocopherol acetate (4000.0) were prepared in microemulsion buffer and transferred to amber glass bottles, in order to protect from light. These solutions were sonicated for 15 min to aid dissolution and kept under refrigeration. An additional stock solution of folic acid (2000.0 μg/mL) containing niacin at 260.0 μg/mL concentration was also prepared.

2.7. Test-mixture standard preparation

Appropriate aliquots from the standard stock solutions were transferred into a 1 mL test tube shaped volumetric amber flask and the volume was completed with microemulsion buffer. The test mixture containing the seven compounds had final concentrations of 200.0 or 400.0 μg/mL on each vitamin. The test mixture was then sonicated for 15 min and injected on the CE instrument.

2.8. Preparation of internal standard solution

An internal standard solution of niacin (260.0 μg/mL) was prepared in microemulsion buffer. The solution was sonicated for 15 min.

2.9. Analytical curve

Aliquots of 80.0, 90.0, 100.0, 110.0, and 120.0 μL, from the standard stock solution of folic acid (2000.0 μg/mL) containing internal standard were transferred into separate 1 mL volumetric amber flasks. Samples were diluted to volume with the internal standard solution. Concentration range from 160.0 to 240.0 μg/mL of folic acid, and 260.0 μg/mL of niacin were obtained. The solutions were sonicated for 15 min and injected on the CE instrument. Each solution was injected in triplicate. Peak area ratios (PAR) (folic acid/niacin) were plotted against the respective concentrations of folic acid.

Table 1
Procedure for the recovery test (standard solution of folic acid added to commercial sample solution)

Folic acid standard solution (μg/mL)	Commercial sample solution[a] (μg/mL)	Final concentration (μg/mL)	
2000.0[b]	2000.0[b]	Folic acid	Internal standard
Aliquots (μL)[c]			
40	40	160.0	260.0
50	50	200.0	260.0
60	60	240.0	260.0

[a] Procedure was performed on commercial samples (tablets of folic acid, laboratory A).
[b] Containing internal standard, niacin, at 260.0 μg/mL.
[c] To 1 mL volumetric amber flasks.

2.10. Sample preparation

Twenty folic acid tablets were finely powdered and an amount corresponding to 50.00 mg of folic acid was weighed, and transferred into 25 mL volumetric amber flasks. Samples were diluted to volume with internal standard solution. The solution was sonicated for 30 min and filtered using a Whatman 40 filter paper, rejecting the first 5 mL filtered portion. An aliquot of 100.0 μL was transferred into 1 mL volumetric amber flask and volume was completed with internal standard solution. The final concentrations were 200.0 and 260.0 μg/mL of folic acid and niacin, respectively. The sample solution were sonicated for 15 min prior to introduction onto the capillary.

2.11. Accuracy

To determine the accuracy of the method, recovery experiments were performed according to procedures endorsed by AOAC International [17]. Folic acid standard solution was added to commercial sample solutions and analyzed by the proposed method, according to the procedure depicted in Table 1.

3. Results and discussion

3.1. Method development

In MEEKC, one of the most commonly used microemulsion formulations contains 0.81% (w/w) octane, 3.31% (w/w) SDS, 6.61% (w/w) butan-1-ol and 89.27% (w/w) phosphate or tetraborate buffer. This microemulsion does not allow fast separations because of the high SDS concentration. As it was mentioned before, due to the high interfacial surface tension between the oil and the aqueous phase, a large amount of surfactant is needed to form droplets. However, high levels of SDS restrict the voltage that can be applied (13–15 kV), since an excessive SDS content leads to high operating currents. Some reports on the applications of MEEKC with the above mentioned octane system presented 60 min separations

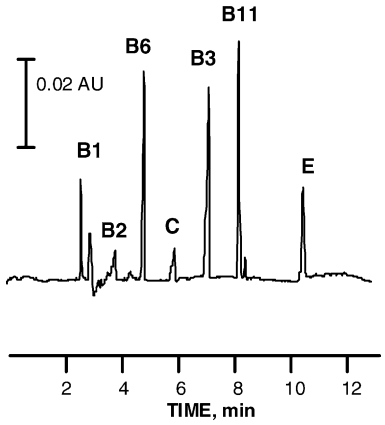

Fig. 2. Electropherogram of a standard mixture of vitamins. Microemulsion composition: 0.5% (w/w) ethyl acetate, 1.2% (w/w) butan-1-ol, 0.6% (w/w) SDS, 15% (v/v) 2-propanol and 82.7% (w/w) 10 mmol L^{-1} tetraborate buffer at pH 9.2. Injection: 0.5 psi/5 s (sample) followed by 0.1 psi/1.5 s (microemulsion buffer). Applied voltage: +28 kV. Direct UV detection at 214 nm. Peaks: B_1 (thiaminium mononitrate), B_2 (riboflavine), B_3 (niacin), B_6 (pyridoxol hydrochloride), B_{11} (folic acid), C (ascorbic acid) and E (α-tocopherol acetate).

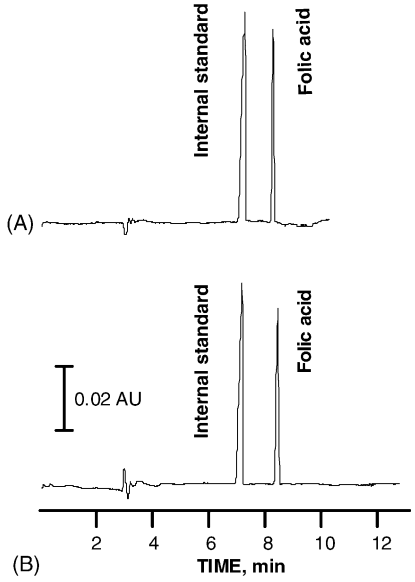

Fig. 3. Analysis of a folic acid tablet formulation: (A) standard solution of folic acid at 200.0 μg/mL; (B) commercial sample containing folic acid at 200.0 μg/mL. In (A) and (B), niacin at 260.0 μg/mL was used as internal standard. Experimental conditions and peak labels as in Fig. 2.

of hydrophobic solutes such as fat soluble vitamins, steroid, and fatty acid derivatives [18–20]. Oils such as ethyl acetate and di-*n*-butyl tartrate have a low interfacial surface tension and require lower SDS concentration than octane to form stable microemulsions. Since the requirements for use of an analytical method in a routine pharmaceutical analysis include a relatively rapid separation, ethyl acetate oil-based microemulsions were used in the present work.

Initial experiments were carry out with aqueous microemulsion buffer containing 0.5% (w/w) ethyl acetate, 1.2% (w/w) butan-1-ol, 0.6% (w/w) SDS and 97.7% (w/w) 10 mmol L^{-1} tetraborate buffer at pH 9.2; the Vitamins B_{11} and B_3 (internal standard) overlapped (not shown). To resolve this problem 2-propanol (organic modifier) was added to the microemulsion and all vitamins were well separated (Fig. 2). The use of propanol had been proposed previously by Sánchez et al. [21] for the octane microemulsion system to conduct the separation of the water-and fat-soluble vitamins. The microemulsion containing 0.5% (w/w) ethyl acetate, 1.2% (w/w) butan-1-ol, 0.6% (w/w) SDS, 15% (v/v) 2-propanol and 82.7% (w/w) 10 mmol L^{-1} tetraborate buffer at pH 9.2 was used for the rest of work. It was found to be stable and no performance degradation was observed during storage for 1 month.

The internal standard (niacin) was used to improve precision. Internal standards are often used to minimize injection volume fluctuations, dilution errors and errors during sample treatment. An internal standard can substantially improve the precision of peak area determinations, especially if the injection error is the dominant source [22]. Fig. 3A illustrates the separation of folic acid from niacin. The CE retention times for the standards folic acid and niacin (IS), were approximately 8.0 and 7.0 min, respectively. The resolution between folic acid and the internal standard as well as the column efficiency were 3.4 and 5.9 × 10^4 plates/m, respectively.

3.2. Method validation

Before a method is routinely used, it must be validated. In the present work, the MEEKC methodology was validated by determining its performance characteristics regarding selectivity, injection precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy [3,23].

3.2.1. Selectivity

The method presented a good separation selectivity for all considered vitamins (Fig. 2). In addition, folic acid and niacin (internal standard) were well separated and easily quantified (Fig. 3).

3.2.2. Injection precision

Ten injections of the mixture (folic acid, 200.0 μg/mL and niacin, 260.0 μg/mL) were performed to demonstrate the injection precision of the system and peak area ratio was calculated. Folic acid gave a repeatability of 1.2% relative standard deviation (R.S.D.) for PAR. The injection precision was therefore considered satisfactory [24].

3.2.3. Linearity, limit of detection and limit of quantitation

To establish the method linearity, peak area ratios (folic acid/niacin) versus concentration data were treated by linear least-square regression analysis [25]. Acceptable coefficients of correlation (0.99 or greater) and an intercept close to the origin should be achieved [22]. The analytical curves

Table 2
Method validation regarding linearity and limits of detection and quantification

Parameter	Statistical data
Concentration range[a] (μg/mL)	160.0–240.0
Intercept	−0.01333
Slope	0.00398
Coefficient of correlation (*r*)	0.9992
Standard error estimate	0.0059
Limit of detection (μg/mL)	2.98
Limit of quantitation (μg/mL)	9.05

[a] Five data points, three replicate injections at each concentration level.

consisted of five data points and three replicate injections at each concentration level were performed. As shown by the statistical data organized in Table 2, the method exhibited excellent linearity ($r > 0.99$) between peak area ratios (folic acid/niacin) and folic acid concentration over the concentration range of 160.0–240.0 μg/mL. The limits of detection and quantitation for folic acid were 2.98 and 9.05 μg/mL, respectively. The criterion used to determine the LOD and LOQ was based on the determination of the slope (*S*) of the calibration curve and the standard deviation of responses (S.D.) in accordance with the formulas LOD = 3.3 S.D./*S* and LOQ = 10 S.D./*S* [23]. The standard deviation of response was determined from the standard error estimate of the regression line.

3.2.4. Precision of the method (repeatability)

Sample solutions were prepared at three concentrations levels (160.0, 200.0 and 240.0 μg/mL) and three determinations for each concentration were performed to establish the intra-assay precision. The repeatability of the method was demonstrated by the mean relative standard deviation for PAR. Data presented in Table 3 indicated a good agreement among the individual test results. The criterion for precision demands a R.S.D. better than 2.0% [24].

3.2.5. Accuracy

Accuracy was calculated as the percentage recovery of a known amount of standard added to the sample [3]. Table 4 shows the accuracy of the method with recoveries for folic acid ranging from 98.08 to 101.72% for the sample. Mean recoveries should be 100 ± 2% at each concentration over the range of 80–120% of the target concentration [24].

Table 3
Method validation regarding precision of the method

Sample concentration[a] (μg/mL)	R.S.D. for PAR (%)
160.0	0.71
200.0	0.41
240.0	0.92
R.S.D.[b] (%)	0.68

PAR: peak area ratio (folic acid/internal standard).
[a] Three determinations at each concentration level.
[b] R.S.D. (nine determinations).

Table 4
Method validation regarding accuracy: recovery test

Standard added to commercial sample (μg/mL)	Standard found (μg/mL)	Recovery (%)[a]
80.0	81.4	101.7
100.0	99.5	99.5
120.0	117.7	98.1

Average of three determinations.
[a] Commercial sample (folic acid tablets) from laboratory A.

Table 5
Assay results for commercial sample of folic acid

Results	Sample (folic acid)
Amount declared (mg/mL)	5.00
Amount found[a] (mg/mL)	5.012 ± 0.009
Purity (%)	100.2
Response factor R.S.D. (%)	0.92

[a] Average of three determinations.

3.2.6. Assay

Sample of folic acid was assayed against a reference standard solution of folic acid. Both solutions were prepared at 200.0 μg/m in folic acid. The results in Table 5 demonstrated that the MEEKC method was suitable for determination of folic acid. The assay was found to be satisfactory, the sample analysed was within tolerance limits (90–115% label claim) and the R.S.D. of the response factor was lower than 1%.

3.2.7. Application of the method to the commercial vitamin formulation

To demonstrate the applicability of MEEKC method under conditions described before, commercial sample tablets containing folic acid were analysed. The results shown in Fig. 3B indicated that the method can potentially be applied to determination of folic acid in pharmaceuticals.

4. Conclusions

A novel MEEKC method for the analysis of folic acid in tablets has been developed and validated with respect to selectivity, injection precision, linearity, limit of detection and quantification, precision and accuracy. The method adequate analytical performance makes it suitable for implementation in pharmaceutical laboratories for the routine analysis of folic acid formulations.

Acknowledgements

The authors wish to acknowledge the Fundação de Amparo à Pesquisa do Estado de São Paulo of Brazil (Fapesp, 00/04414-4, 02/07390-4) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil (CNPq, 301201/94-3) for financial support and fellowships.

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