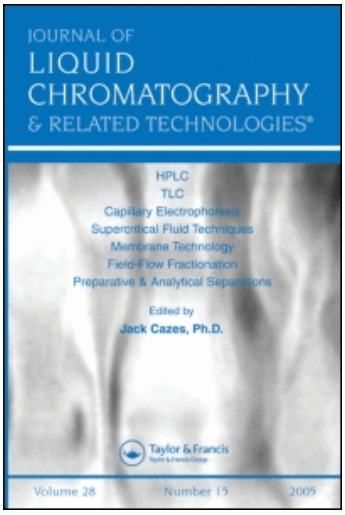

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Determination of *S*-Adenosyl-*L*-methionine in Dietary Supplement Preparations by Capillary Electrophoresis

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ABSTRACT

A method for the quantitative determination of *S*-adenosyl-*L*-methionine (SAM) in dietary supplement preparations using capillary zone electrophoresis (CZE) is described. Linearity was observed in a concentration range of 10–200 μg mL^{-1} with $r = 0.9997$. The limit of detection was 0.5 μg and the limit of quantification 2 μg. The intra-day and the inter-day precision was in the range of 0.52–0.89 and 1.15–1.60% RSD, respectively. The mean recovery determined by spiking with known amounts of SAM was 97.9%. Furthermore, stability investigations of SAM were carried out using this method.

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Key Words: *S*-Adenosyl-*L*-methionine; Dietary supplement; Capillary electrophoresis.

INTRODUCTION

Dietary supplements became very popular in recent years. One compound that attracted increasing attention is *S*-adenosyl-*L*-methionine (SAM, Fig. 1). *S*-adenosyl-*L*-methionine plays an important role in human physiology. It acts as a methyl donor and is a cofactor for COMT, PNMT, and other enzyme-catalyzed methylations.[1,2] Such methyl donors are believed to have activity in preventing heart disease, stroke,[3–5] cancer,[6,7] and neurological disorders.[8] *S*-adenosyl-*L*-methionine has been found to show a cancer preventing activity in animals.[9] Furthermore, it found application as an antidepressive drug and for treatment of migraine.[10–17]

S-adenosyl-*L*-methionine is a rather unstable compound. Therefore, the development of methods for quantitative determination and stability control of SAM in pharmaceutical and dietary supplement preparations is of high interest. Several publications deal with the determination of SAM and its metabolites in biological samples using HPLC.[1,10,18–21] For the determination of SAM in tablets, two HPLC-methods have been recently reported.[22,23]

Capillary electrophoresis has turned out to be a simple and fast approach for the analysis of pharmaceuticals and compounds of biological relevance. To our knowledge, no CE method exists to date for the analysis of SAM.

This work deals with the development of a rapid and simple method, which allows the quantitative determination of SAM in dietary supplement preparations and stability control using CE.

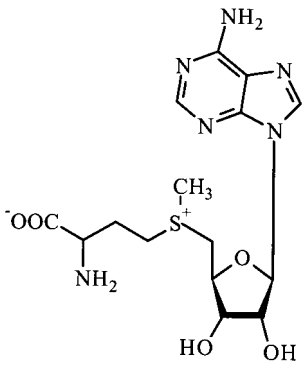

Figure 1. Chemical structure of SAM.

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EXPERIMENTAL

Apparatus

All analyses were performed on a SpectraPHORESIS 1000 (Spectra Physics Inc., San Jose, CA) capillary electrophoresis system equipped with a diode array detector and Software PC1000, Version 3.0 (Thermo Separation Products, Fremont, CA).

The system was flushed with water (3 min), 0.1 M sodium hydroxyde solution (5 min) and water (3 min) prior to use. Separations were carried out using a fused silica gel capillary, 75 μm I.D., 44 cm length (38 cm to detector). UV detection was set to 208 nm. All experiments were done using a voltage of 10 kV.

Chemicals and Reagents

Chemicals were of analytical grade unless indicated otherwise. *S*-adenosyl-*L*-methionine-chloride and phosphate salts were from Sigma-Aldrich (St. Louis, MO).

Methods

Samples were injected hydrodynamically for 6 s. Electrolyte solutions were prepared from sodium dihydrogenphosphate and disodium hydrogenphosphate and adjusted to pH 7, or, in case of pH 4.3 a 50 mM solution of sodium dihydrogen phosphate was used. Electrolyte was degassed and filtered through a 0.2 μm pore size filter (Schleicher and Schuell, Dassel, Germany). All separations were performed at ambient temperature.

Standard Solutions and Calibration Curve

A stock solution of 5 mg SAM-chloride in 5 mL 50 mM phosphate buffer pH 7 was prepared. This solution was stored below 4°C. For the preparation of the calibration curve aliquots between 75–1500 μL were taken and diluted to 5 mL. A calibration curve was prepared by calculating the amount of intact SAM from the relation of the peak areas of SAM to the sum of the peak areas including the degradation products.

Analysis of Tablets

Ten tablets were weighed and finely pulverized. An amount of this powder equivalent to about 200 mg SAM was weighed and transferred to a 50 mL

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Erlenmeyer flask and extracted subsequently with 3 portions of 15 mL 50 mM phosphate buffer pH 7. After filtration, extracts were united and diluted to a total volume of 50 mL in a volumetric flask. One milliliter of this solution was further diluted to 50 mL prior to injection.

RESULTS AND DISCUSSION

Separation Studies

Various electrolytes were checked in order to separate SAM and the degradation products. Using a 50 mM phosphate buffer pH 7 as electrolyte, two peaks appeared with migration times of about 4.0 and 4.8 min, whereby the first one corresponded to SAM and the second to degradation products, which co-migrate under these conditions. When a phosphate buffer pH 4.3 was used, migration times increased and three peaks were observed (Fig. 2). The nature of the degradation products was not investigated, however, according to the literature the main degradation products of SAM are believed to be *S*-adenosyl-homocysteine (SAH) and 5-deoxy-5-methylthioadenosine (DMTA).[23]

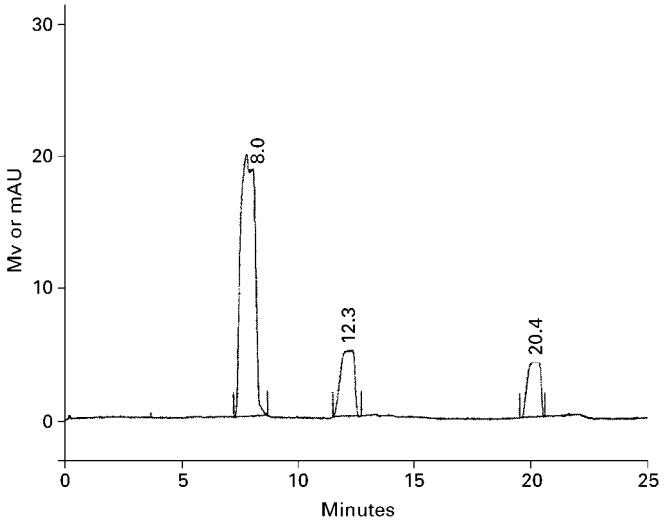

Figure 2. Electropherogram of a commercially available sample of SAM using phosphate solution pH 4.3. Conditions: Fused silica capillary, 75 µm ID, 42 (38) cm. Electrolyte: 50 mM phosphate, pH 4.3. Voltage: 10 kV, detection at 208 nm. Peak 1: SAM, peaks 2 and 3: degradation products.

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Stability Studies

S-adenosyl-*L*-methionine has been found to be a rather unstable compound. The supplier claims for the SAM-chloride reference compound (Sigma) a 80–90% purity at origin, but takes into account a decomposition of up to 10% per day if stored at +25°C. A storage at less than −20°C is recommended. When we injected a freshly prepared solution of this product, a content of about 65% intact SAM was measured. After the product was stored below −20°C no significant loss was observed.

Kinetic studies were performed with the solutions of SAM-chloride reference compound stored at +4°C and +25°C for 7 days; results are shown in Fig. 3: A drastic degradation was observed with solution of SAM in water at 25°C.

It was found that degradation increases with increasing pH.

Repeatability of Migration Time and Peak Area

Five subsequent injections were made of a standard solution containing 100 μg mL^{-1} to check repeatability of migration time and peak area.

The data are shown in Table 1.

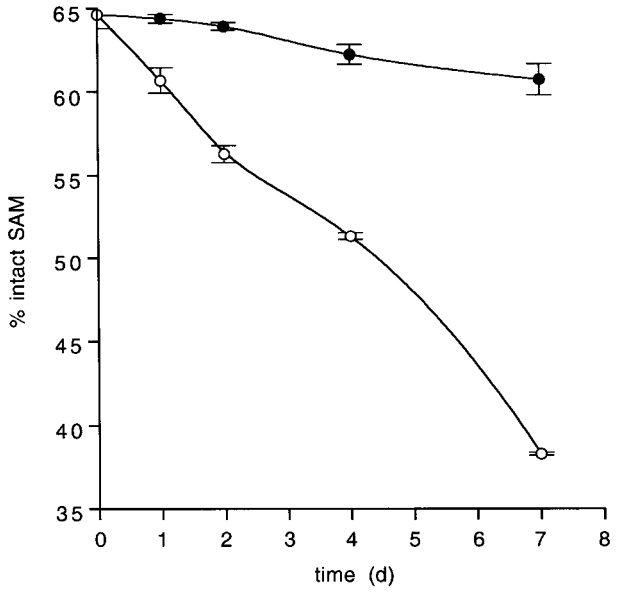

Figure 3. Kinetic studies of SAM. Solution stored at 4°C (●). Solution stored at 25°C (○).

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Table 1. Precision of migration time and peak area ($n = 5$).

t_{mig} (min)	4.03 ± 0.07
RSD (%)	1.57
Peak area	2525254 ± 472.37
RSD (%)	0.18

Calibration

Taking into account the instability of SAM and the fact that no pure SAM reference samples are available, the calibration using such standards would lead to too high results in the analysis of samples. Therefore, corrections in calibration have to be made.

For quantitative determination a phosphate buffer pH 7 was used as electrolyte. Under these conditions the degradation products co-migrate. Assuming that the difference in UV-absorption between the intact SAM and the mixture of the degradation products is neglegible, calibration curves using SAM standard solutions can be prepared by calculating the amount of intact SAM from the relation of the peak areas of SAM to the sum of the peak areas including the degradation products. Using this calibration curve, the amount of intact SAM in the tablets can be calculated. Calibration curves were prepared newly every day by injecting five standard solutions of SAM at different concentrations.

The linear range was found to be 10–200 $\mu g\,mL^{-1}$ with an average correlation coefficient of 0.9997 (Table 2).

The LOD and LOQ were estimated in accordance with the baseline noise. The baseline noise was evaluated by recording 10 times the peak width. The LOD was obtained as a sample concentration that caused a peak with a height three times the baseline noise level and the LOQ was calculated as 10 times the baseline noise level (Table 2).

Table 2. Linearity and detection limits (calculated as intact SAM).

Slope	3866.2
Intercept	2655.2
Linear range	10–200 $\mu g\,mL^{-1}$
R	0.9997
LOD	0.5 $\mu g\,mL^{-1}$
LOQ	2 $\mu g\,mL^{-1}$

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Table 3. Purity control and intra-day and inter-day precision.

Nominal conc.[b] ($\mu g\,mL^{-1}$)	Intra-day ($n = 5$)		Inter-day ($n = 15$)	
	Found[a] ($\mu g\,mL^{-1}$)	RSD (%)	Found[a]	RSD (%)
25	16.27 ± 0.146	0.89	16.28 ± 0.302	1.15
150	97.05 ± 0.507	0.52	96.77 ± 1.500	1.60

[a]Calculated as intact SAM.
[b]Concentration of standard solution of a commercially available reference compound.

Purity Control of a Commercially Available Reference Compound and Precision of the Method

In order to check the repeatability, replicate injections of standard solutions of a commercially available reference compound at two different concentrations were made (intra-day precision). The evaluation was done by means of the calibration curve prepared as described above. The inter-day precision was examined over a period of three consecutive days, preparing the solutions freshly every day. The results are shown in Table 3.

The content of this reference compound was found to be about 65%.

Quantitative Determination of SAM in Tablets

For the quantitative determination of SAM in the tablets, 10 tablets were pulverized and aliquots corresponding one tablet were extracted. The amounts found in the tablets were slightly higher than the claimed amount; this might be due to an overdose to cover the loss by decomposition (Table 4). Figure 4 shows electropherograms of a SAM standard solution (A) and a tablet extract (B).

Table 4. Quantitative determination of SAM in tablets.

Claimed amount (mg)	200
Calculated as intact SAM	
Found ($n = 5$) (mg)	209.6 ± 5.78
RSD (%)	2.75
SAM including degradation products	
Found ($n = 5$) (mg)	233.6 ± 6.44
RSD (%)	2.75

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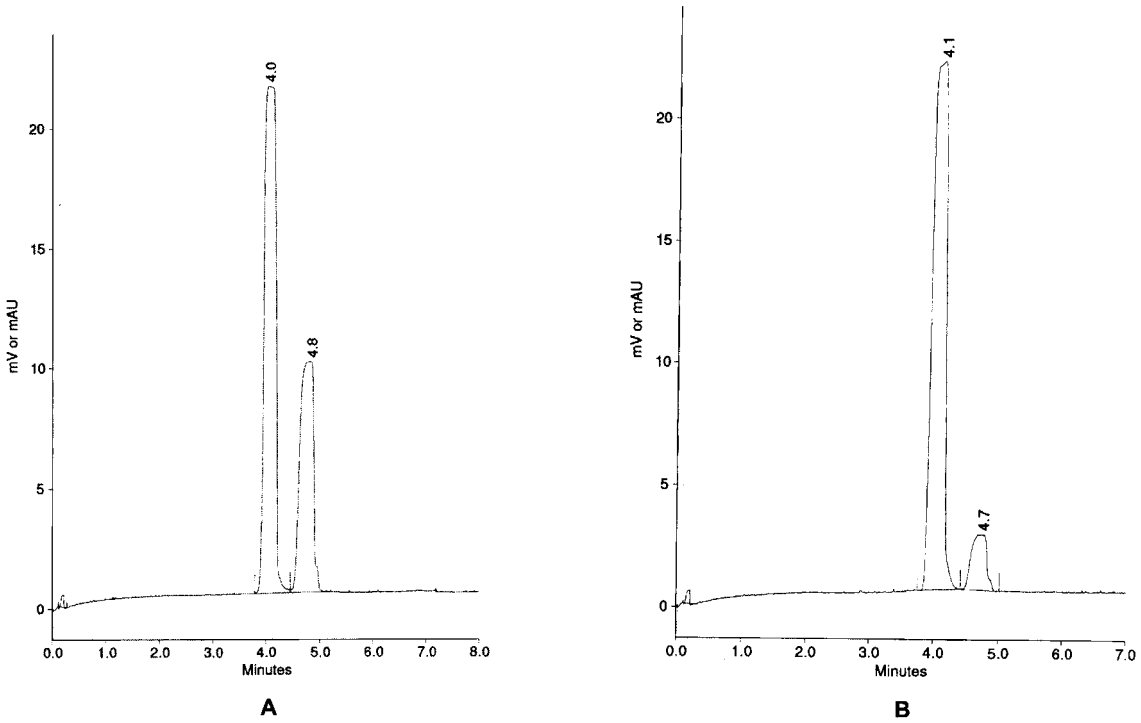

Figure 4. Electropherogram of SAM using phosphate solution pH 7.0. Conditions: Fused silica capillary, 75 μm ID, 42 (38) cm. Electrolyte: 50 mM phosphate, pH 7.0. Voltage: 10 kV, detection at 208 nm. (A) SAM standard solution; (B) tablet extract. Peak 1: SAM, peak 2: degradation products.

Table 5. Recovery studies.

Added amount	40 mg
Found[a]	39.15 ± 0.843 mg
Recovery (%)	97.9
RSD (%) ($n = 5$)	2.15

[a]Calculated as intact SAM.

For the calculation of the total amont of SAM, including the degradation products present in the tablet (corresponding to the originally introduced amount of SAM), a calibration curve using the sum of the peak areas of SAM and degradation products was prepared.

To control the variation of the content in the single tablets, five different tablets were extracted and the amount of SAM determined. The dose deviation was found to be 3.29% RSD.

For the determination of the recovery, the preparation was spiked with known amounts of SAM. The results are given in Table 5.

Surprisingly, the degradation in the tablets was found to be lower compared to SAM standard (about 10%). It is assumed that microencapsulated SAM was used for the preparation.

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