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HPLC analysis of *S*-adenosyl-L-methionine in pharmaceutical formulations

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A validated analytical method of *S*-adenosyl-L-methionine (SAM) in pharmaceutical preparations by reversed phase high performance liquid chromatography (RP-HPLC) is described. The compound is separated by a 2.1 mm × 15 cm, 5 μm-Discovery C_{18} column with isocratic elution. The effect of different anionic surface active agents with different molarity on the separation was studied. A direct relationship between the molarity of the surface active agents and the capacity factor (k′) was found. The limit of detection was 0.49 mmol/ml and the linearity was r = 0.999 in the concentration range 20–100 μg/ml. Inter- and intra-assay variation was determined for three selected concentrations (20, 60, 100 μg/ml) by calculating the analytical recoveries with a range of 97.0–99.9%. The procedure was also suitable to check the stability of *S*-adenosyl-L-methionine in solution at room temperature.

1. Introduction

The energy and methylation potentials of cells depend on and are interrelated through adenine derivatives. Energy homeostasis depends on the levels of adenine nucleotides, and methylation reactions depend on the levels of *S*-adenosyl-L-methionine (SAM) [1].

S-adenosyl-L-methionine (SAM), is an endogenous compound that plays a central role in many biochemical processes [2, 3]: as the labile methyl group donor in numerous transmethylation reactions, as the amino propyl group donor in polyamine biosynthesis, and as precursor in the synthesis of cysteine [4].

Pharmacologically, SAM is administered as a treatment for clinical depression [5–9]. Physiological methyl donors have been associated with a decreased risk of colon and liver cancer [10, 11] and SAM itself is a cancer chemopreventive agent in animals [12]. These methyl donors also appear to exert a role in preventing heart disease, stroke [13–15], cancer and neurological disorders [16].

This paper describes the analysis and stability of SAM in pharmaceutical tablet formulations by means of reversed phase HPLC, and the effect of several anionic surface active agents on the separation.

2. Investigations, results and discussion

The chromatographic procedure described allows the analysis of SAM with a simple isocratic HPLC system, which

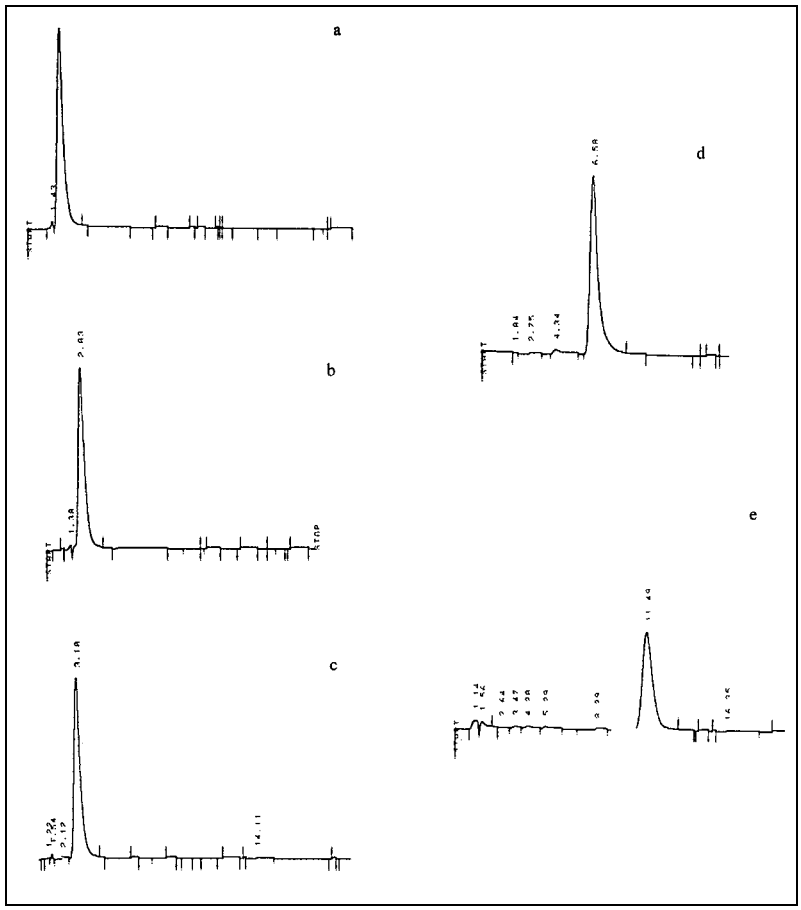

Fig.:
Effect of the surface active agents on the SAM separation (using 5 mM for each one), a = without surface active agent, b = 1-pentane sulfonate, Na salt, c = 1-hexane sulfonate, Na salt, d = 1-heptane sulfonate, Na salt, e = 1-octane sulfonate, Na salt

has a clear advantage over previously published methods which involved a gradient elution profile [17, 18]. The calibration curve reflects linearity in the concentration range of 20–100 μg/ml with a correlation coefficient $r = 0.999$.

One of the goals of this report is to study the effect of several anionic surface active agents with different molarity on the SAM analysis. SAM is relatively polar compound and as a consequence it displays relatively short retention time under reversed-phase chromatographic conditions even if very low concentrations of organic modifier are used [19, 20].

To increase the retention time of the positively charged SAM, a reversed-phase chromatographic method was suggested using heptane sulfonic acid in an acidic mobile phase [21–24]. The Fig. presents the effect of the anionic surface active agents namely, 1-pentane sulfonate, 1-hexane sulfonate, 1-heptane sulfonate, 1-octane sulfonate. It was of interest to notice that SAM had a much longer retention time with increasing the alkyl chain of the surfactant used and its molarity in the mobile phase as the ion-pair complex formed between SAM and the anionic surfactant will be retained longer on the C_{18} stationary phase leading to an increase of the capacity factor (k') as shown in Table 1.

We choose 1-heptane sulfonate sodium salt as a representative example of serial molarity (0,1, 2, 5, 10, 15 mM). Table 2 indicates that the capacity factor (k') did increase with increasing the molarity of the surface active agents.

The limit of detection for the assay was estimated to approximately 0.49 mmol/ml (S/N = 3). Inter-assay variability (reproducibility) was assessed singly in six replicate runs over the concentration range 20, 60 and 100 μg/ml, as shown in Table 3, the coefficient of variation ranged from 0.4–3.64%. The analytical recoveries ranged from 97–99.3%. The results were similar for the intra-assay variation, Table 4, with analytical recoveries ranging from 98.8–99.9% and coefficients of variation being 0.92–21.7%.

Five tablets of a SAM formulation (SAM-e, containing 200 mg SAM per tablet) were grinded to a fine powder. An amount of powder equal to one tablet was weighed and dissolved in distilled water, sonicated for 30 min and centrifuged. The clear supernatant solution (1 ml) was taken from the stock solution and diluted with the mobile phase to form a solution parallel in its concentration to that of the standard solution (100 μg/ml).The recovery was found to be 92%. It is of interest to note that the solution of SAM in the mobile phase was found to be stable at room temperature for more than 13 days.

Table 1: Effect of the alkyl chain on the capacity factor (k')

Surface active agents	Capacity factor (k')
No surface active agent	0.057
1-Pentane sulfonate, Na salt	0.29
1-Hexane sulfonate, Na salt	0.93
1-Heptane sulfonate, Na salt	3.08
1-Octane sulfonate, Na salt	7.48

Table 2: Effect of the molarity of 1-heptane sulfonate, Na salt on the capacity factor (k')

Molarity (mM)	Capacity factor (k')
0	0.057
1	0.5
2	1.25
5	3.04
10	3.29
15	4.36

Table 3: Intra-assay precision and accuracy of SAM (n = 6)

Conc. (μg/ml)	Theoretical AUP(10^7)	Experimental AUP(10^7)	SD	CV (%)	Analytical Recovery (%)
100	4.48	4.47	0.966	21.7	99.9
60	2.57	2.54	0.324	1.28	98.6
20	0.88	0.87	0.008	0.92	98.8

(Mobile phase used was 0.1 M sodium acetate, 5 mM heptane sulfonate Na salt, pH 4.5 with a glacial acetic acid, 4.2% acetonitrile)

Table 4: Inter-assay precision and accuracy of SAM (n = 6)

Conc. (μg/ml)	Theoretical AUP (10^7)	Experimental AUP(10^7)	SD	CV (%)	Analytical Recovery (%)
100	4.48	4.45	0.0189	0.4	99.3
60	2.57	2.54	0.025	1	98.6
20	0.88	0.86	0.0311	3.64	97.0

(Mobile phase used was 0.1 M sodium acetate, 5 mM 1-heptane sulfonate Na salt, pH 4.5 with glacial acetic acid, 4.2% acetonitrile)

3. Experimental

3.1. Apparatus

A Waters HPLC system was used, consisting of a Waters 501 solvent delivery pump, a Rheodyne model 7125 injector and a Lambda Max model 481 LC spectrophotometer UV detector (Waters Milford, MA, USA) coupled with a HP 3394 integrator (Hewlett Packard, Avondale, PA, USA). Separation is achieved on a reversed-phase 2.1 mm × 15 cm, 5 μm-Discovery C_{18} column (Supelco, Bellefonte, PA, USA). The mobile phase consisted of 0.1 M sodium acetate, surface active agent with specific molarity, adjusted to pH 4.5 with glacial acetic acid, to which 4.2% acetonitrile was added (Table 1). A stock solution of SAM was prepared in a concentration of 0.1 mg/ml, using the mobile phase as a solvent.

3.2. Chemicals

Sodium acetate, HPLC grade was obtained from Fisher (Springfield, NJ, USA), sodium salt of 1-pentane sulfonate, 1-hexane sulfonate, 1-heptane sulfonate, 1-octane sulfonate, HPLC grade were obtained from Regis (Morton Grove, IL, USA). Glacial acetic acid was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), acetonitrile, HPLC grade, purchased from Fisher (Springfield, NJ, USA). HPLC grade water was used all throughout the experiment prepared from Milli Q apparatus (Millipore, Bedford, USA).
S-adenosyl-L-methionine (SAM) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). The pharmacutical formulation SAM-e tablet (containing 200 mg of SAM) was obtained from a commercial source.

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