Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 449–456
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Evaluation of chemical and diastereoisomeric stability of *S*-adenosylmethionine in aqueous solution by capillary electrophoresis

Claudia Desiderio [a,*], Rosaria A. Cavallaro [b], Antonella De Rossi [a], Fabrizio D'Anselmi [b], Andrea Fuso [b], Sigfrido Scarpa [b]

[a] *Istituto di Metodologie Chimiche, Consiglio Nazionale delle Ricerche, CNR, Area della Ricerca Roma 1, Via Salaria km 29,300, 00016 Monterotondo Stazione, Rome, Italy*
[b] *Dipartimento di Chirurgia "P. Valdoni", Università di Roma "La Sapienza", Via A. Scarpa 14, 00161 Rome, Italy*

Received 9 December 2004; received in revised form 27 January 2005; accepted 28 January 2005
Available online 17 March 2005

Abstract

Capillary electrophoresis was used for monitoring the stability of *S*-adenosylmethionine in aqueous solution under different conditions of storage and incubation used for "in vitro" and "in vivo" experiments, by evaluating both the entity of degradation and the possibility of epimerization at the sulfonium group. The determination of *S*,*S*-*S*-adenosylmethionine in presence of its *R*,*S*-epimer and degradation products was performed in uncoated capillary of 50 μm ID using 150 mM sodium phosphate buffer at pH 2.5. The analyses were performed in short or long-end injection modes depending if a fast monitoring of the degradation products or the evaluation of the diastereoisomeric ratio were carried out, respectively. In the long-end injection mode the baseline separation of *S*-adenosylmethionine diastereoisomeric forms and degradation products was obtained in less than 10 min with efficiency values in the range of 172,520-311,439 number of theoretical plates per meter. The results showed that freezing was the optimum storage mode for *S*-adenosylmethionine aqueous solutions preserving from degradation and diastereoisomeric ratio alterations. Under incubation conditions at 38 °C during 14 days period *S*-adenosylmethionine showed a strong degradation and the formation of three main increasing degradation products. After 7 and 14 days only the 52% and 32% of the initial drug concentration were available and the active *S*,*S*-*S*-adenosylmethionine form was the most affected.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Capillary electrophoresis; *S*-adenosylmethionine; Drug; Stability; Degradation

1. Introduction

S-adenosylmethionine (SAM) is the primary methyl donor present in all living organisms involved in the methylation of target molecules as DNA, proteins, lipids and polyamines synthesis [1,2] (Fig. 1). Abnormalities in SAM metabolism has been well recognized in liver diseases and in various neurological disorders, e.g. Alzheimer disease (AD) and cardiovascular disease [3–5]. SAM exists in two diastereoisomeric forms, the *S*,*S*- and the *R*,*S*-*S*-adenosylmethionine, where the designation refers to the stereochemical configurations of the sulphur and the α-carbon, respectively. Only the *S*,*S*-form is the biologically active: in mammals there is only a minor fraction of the *R*,*S*-SAM [6].

The pharmaceutical preparations of SAM have been increasingly used for the treatment of liver disease as well as neurological and affective disorders but the main problem is represented by its high chemical and diastereoisomeric instability at specific temperatures and pH values [7–9]. There are different mechanisms for SAM degradation: the cleavage to 5′-methylthioadenosine (MTA) and homoserine lactone, the hydrolysis to adenine and *S*-(5′-deoxy-ribosyl)-L-methionine and the epimerization to the biologically inactive *R*,*S*-SAM (see Fig. 2 for the molecular structures). Several efforts have been recently made in commercialising and synthesising

* Corresponding author. Tel.: +39 690672683; fax: +39 690672269.
E-mail address: claudia.desiderio@imc.cnr.it (C. Desiderio).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.01.041

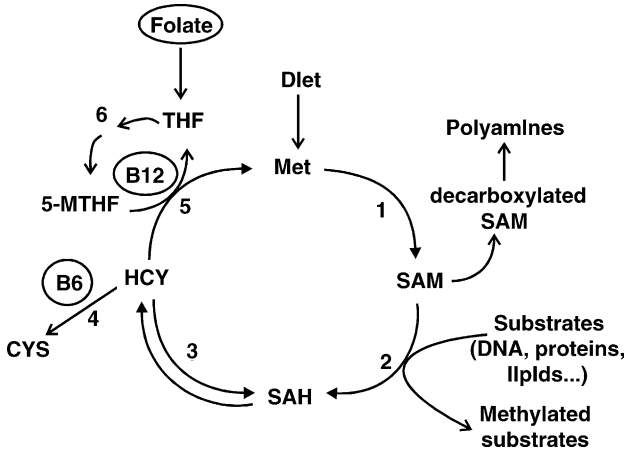

Fig. 1. SAM/HCY cycle. Abbreviations: Met, methionine; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; HCY, homocysteine; CYS, cystathionine; THF, tetrahydrofolate; MTHF, methyltetrahydrofolate; B12, vitamin B12; B6, vitamin B6; 1, methionine adenosyltransferase (MAT); 2, Methyltransferase(s); 3, SAH hydrolase; 4, cystathionine-β-synthase (CBS); 5, methionine synthase; 6, methylenetetrahydrofolate reductase (MTHFR).

SAM salts formulations with higher stability containing larger anions, as e.g. *p*-toluensulfonate anion or amidic derivatives of taurine with fatty acids [10–13].

The pharmaceutical preparations produced using the fermentation technology contain the *R*,*S*-unnatural diastereoisomer in concentrations ranging from 20 to 40% of the total SAM content. Due to the possible interconvertion of the active form into the inactive one and to the high SAM instability, the availability of analytical methodology able to monitor in short time the formation of degradation products and to evaluate the variation of the diastereoisomeric ratio in pharmaceutical preparations is highly requested.

Very few papers deal with stability studies of SAM diastereoisomers by using chromatographic methods [6] or colourimetric assay [14]. In fact, although the analytical determination of SAM was mainly reported by chromatographic methods [15–18], most of them were not able to distinguish the *S*,*S*- from the *R*,*S*-stereoisomers. Recently, capillary electrophoresis (CE) was successfully used for analysing SAM and metabolites [19–21] and studying SAM stability [22] but only two papers [19,20] showed a double peak for SAM attributed to the presence of *R*,*S*-SAM diastereoisomer.

Capillary Electrophoresis, a relatively recently developed miniaturized analytical technique, is characterized by the high resolution power and separation efficiency together with versatility and short analysis time. The high speed of separation and the minimum use of reagent and samples make CE technique particularly suitable for fast monitoring of drug solutions and biological samples.

In this work the high separation efficiency of CE was used for the first time for investigating the stability of SAM in aqueous solution under different storage modes by monitoring both its diastereoisomeric ratio and the formation of degradation products.

The results will provide useful informations for the optimum treatment and storage of SAM salts and solutions and for the development of “in vitro” and “in vivo” experiments, like gene methylation studies [23–25], involving SAM administration.

A comparison between liquid chromatography (HPLC) and CE analytical methodologies for studying SAM stability is also reported and discussed.

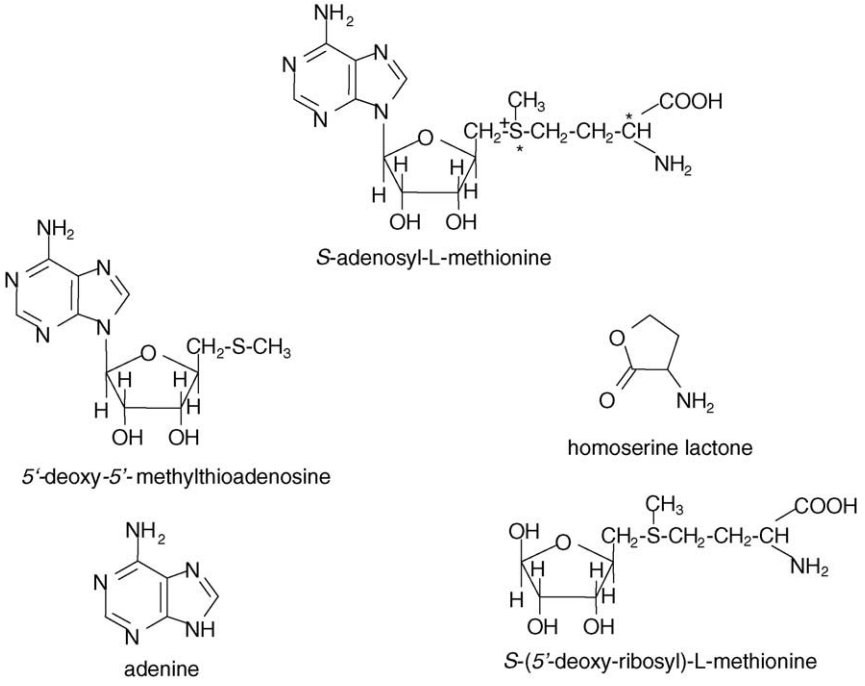

Fig. 2. Chemical structures of *S*-adenosylmethionine and its degradation products.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide anhydrous pearls and orthophosphoric acid (85%, v/v) was purchased from Carlo Erba (Milan, Italy). *S*-adenosylmethionine (SAM) pharmaceutical preparation for injection marketed as Samyr® was from Knoll and contained SAM 1,4-butanedisulfonate and mannitol as excipient. Pindolol was used as reference compound and was purchased from Sigma (St. Louis, MO, USA). Concentrated and diluted analyte solutions were prepared in MilliQ water (Waters, Milford, MA, USA).

Acetonitrile and potassium dihydrogenphosphate were from Sigma (St. Louis, MO, USA).

2.2. Apparatus

Experiments were performed on Agilent Technologies (Waldbronn, Germany) capillary electrophoresis automated apparatus equipped with external nitrogen pressure and diode array UV detector selecting 195 nm as the output wavelength. The separation capillary was 50 μm ID, 375 μm OD, silica fused capillary (Composite Metal Services Hallow, Worcs., UK) of 64.5 cm total length and effective lengths of 56 or 8.5 cm depending if the long or the short end injection modes were used, respectively. The capillary was air thermostated at 25 °C. The applied separation voltage was 30 kV. The analytes were injected at the anodic end by applying pressure of 3 bar × 0.02 min followed by 2 bar × 0.01 min of running buffer to prevent sample loss at voltage application and improve system precision. When the short end injection was applied simply both the polarity and the sample injection end were reversed (negative polarity) inside the same separation capillary.

The running buffer (BGE), 150 mM sodium phosphate pH 2.5, was prepared by titrating 150 mM orthophosphoric acid water solution with sodium hydroxide before taking the solution to the final volume.

The HPLC measurements were carried out using a Varian HPLC system (Varian, Walnut Creek, CA, USA) equipped with a Prostar 210 pump and a UV detector (Prostar 320) set to 254 nm.

Remote control of the HPLC system, data acquisition and calculation of peak areas were performed via computer based data-system (Varian Star 5.3). The samples were injected through an injection valve with a 20 μl sample loop. The mobile phase was pumped at a flow-rate of 1.00 ml/min. The samples were injected onto the reverse-phase column HiRPBC18 (HIRPB-250A, length 250 mm, ID 4.6 mm, 5 μm, Hichrom, Reading, Berkshire, UK). The mobile phases were (pump A) 0.05 M KH_2PO_4, pH 3.5 and (pump B) acetonitrile using the following gradient elution: step (1) time 0 min, 92% A, 8% B, duration 6 min; step (2) time 15 min, 70% A, 30% B; step (3) time 25 min, 60% A, 40% B; step (4) time 35 min, 90% A, 10% B, duration 15 min (riequilibrating step); step (5) return to step (1).

A Finnigan ThermoQuest Navigator mass spectrometer (MassLab Group, Manchester, UK) equipped with an electrospray interface and a single quadrupole was used in trying to characterize the SAM degradation products during the 14 days storage at 38 °C. The mass spectrometer was operating in positive ion mode of ionisation applying a capillary voltage of 3.5 kV and a skimmer cone voltage of 15 V. Mass spectra were collected in the *m*/*z* range 100–500 using a mixture of methanol/ammonium acetate buffer pH 4 (60:40) as delivering solvent. The source temperature was 150 °C.

2.3. SAM solution preparation

A volume of 5 ml of a 50 mM SAM aqueous solution (solution pH 2.6) was prepared from the pharmaceutical preparation Samyr® and divided into four aliquots of 1 ml volume, namely solution I, II, III and IV, stored and analysed as described below:

- Solution I: stored for 3 days at room temperature and analysed each day three times during the day (I run:10 am; II run: 2 pm; III run: 4 pm).
- Solution II: stored at −18 °C for 2 days and analysed three runs per day each time after freezing/thawing procedures.
- Solution III: stored at −18 °C for 1 day and then thawed at room temperature and analysed three times as solution I.
- Solution IV: stored at −18 °C for 3–4 days and then analysed three times as solution I.

Before injection into CE each solution was differently diluted 1:100 (concentrated solution) and 1:1600 (diluted solution) and differently analysed in short- and long-end injection methods, respectively. These two methods differ only in the length of the capillary used for the separation (see the experimental section for further details). The diluted solution was analysed using a separation capillary length of 56 cm (long-end injection method) allowing within 7 min the separation of the SAM diastereoisomeric forms with 1.16 resolution (R_s) value. The concentrated solution was instead analysed in short end injection mode (8.5 cm capillary effective length) allowing a very fast monitoring (total analysis time less than 2 min) of the formation of SAM degradation products.

3. Results and discussion

In this paper capillary zone electrophoresis was employed to monitor the stability of aqueous solution of SAM pharmaceutical preparation under different modes of storage and during 14 days of incubation at 38 °C. Two different series of experiments were arranged (i) to assess the chemical stability of SAM aqueous solutions at room temperature and after several freezing/thawing cycles and (ii) to evaluate the degree of degradation of the SAM aqueous solution incubated at 38 °C

during 14 days, following the conditions used for "in vivo" experiments. In both the experiments the aim was to investigate and identify the formation of degradation products and to monitor the variation of *S*,*S*-/*R*,*S*-SAM diastereoisomeric ratio.

The high resolution power of CE allowed, under the suitable experimental conditions, to obtain the separation of SAM diastereoisomers in free zone electrophoresis without additives or separation media to the running buffer, the *S*,*S*-diastereoisomer migrating faster. The electrophoretic runs were performed in 150 mM sodium phosphate buffer pH 2.5 in uncoated capillary of 64.5 cm of total length (56 cm effective separation length) applying 30 kV voltage. Under these experimental conditions the SAM was positively charged migrating towards the cathode in the same direction of the electroosmotic flow (EOF). The use of an acidic buffer at high concentration strongly reduced the dissociation of the silica silanols on the capillary wall minimizing both the EOF and the adsorption of positively charged compounds improving the peak shape and separation efficiency. Furthermore, the high buffer ionic strength maximized the electrophoretic mobility differences between the diastereoisomers allowing their resolution without the addition of any additive to the separation media. The use of as long capillary as 64.5 cm limited the current increase due to the high buffer concentration allowing however the application of high voltages to speed the separation.

3.1. Study of SAM chemical stability under different storage modes

Due to the poor chemical stability of SAM salts in pharmaceutical formulations at room temperature and in the presence of humidity, different experiments were performed to evaluate its degradation rate and diastereoisomeric stability in aqueous solutions under the temperature values normally used for storage, e.g. room temperature (+21 °C), +4 and −18 °C, and after repeated freezing/thawing procedures.

Fig. 3 shows the electropherograms of the diluted and concentrated solution I (panels a and b), respectively, analysed

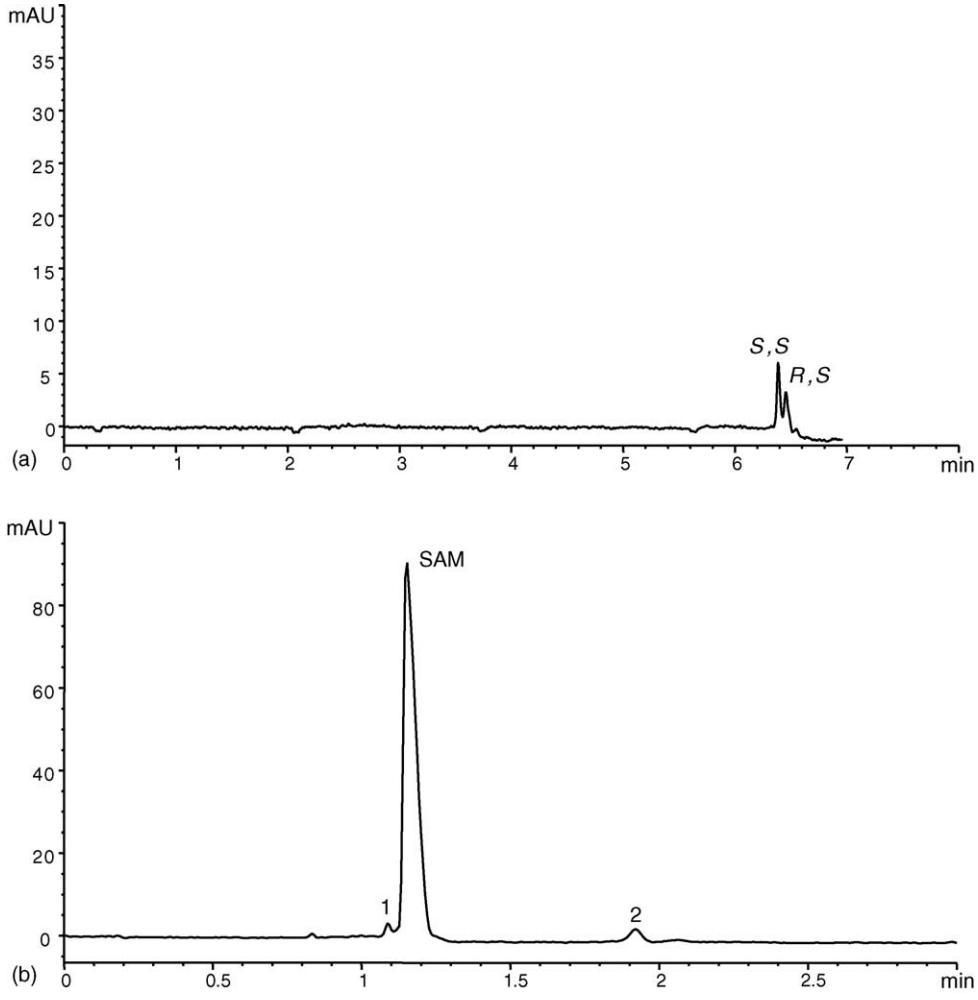

Fig. 3. Analysis of diluted and concentrated SAM aqueous solution by (a) long-end and (b) short end capillary zone electrophoresis, respectively. Buffer: 150 mM sodium phosphate buffer pH 2.5. Capillary: silica fused 50 μm ID × 64.5 total length. Applied voltage 30 kV. For other experimental conditions see the text. *S*,*S*: active *S*,*S*-SAM diastereoisomeric form; *R*,*S*:*R*,*S*-SAM diastereoisomeric form; 1 and 2, pharmaceutical preparation impurities.

Table 1
Comparison of the relative content (area % values) of SAM (total content), SAM diastereoisomers and degradation products (compound 1 and 2) in aqueous solution during 3 days storage at room temperature[a]

Compound	0 Time[b]	Day 2[b]	Day 3[b]
1	1.70	2.53	3.84
2	3.15	7.61	9.85
SAM	95.15	89.86	86.31
S,S/*R,S*	1.63	1.60	1.45

[a] Solution I, for details see the Section 3.
[b] Values of the II run of each day.

in long and short end injection methods at the beginning of the experiments (zero time). In the diluted solution it was not possible to detect the degradation products due to their low concentrations. They were instead clearly visible when the concentrated solution was injected (panel b). The analysis of the concentrated solution I at zero time showed the presence of two other compounds, namely 1 and 2, in addition to the main SAM peak. We observed a not significant variation in SAM, 1 and 2 relative areas in solutions III and IV after either 1 day or 3–4 days of freezing (storing at −18 °C) (data not shown). Therefore in frozen storage the SAM content seemed well preserved and the formation of degradation products prevented.

Table 1 describes the relative area % of compounds 1 and 2 and SAM together with the *S,S*-/*R,S* diastereoisomeric ratio during storage at room temperature for three days (solution I). The SAM concentration progressively decreased from the initial value of 95.15% (relative area) to 89.86% after 2 days and 86.31% after 3 days simultaneously to the increase of the relative areas of the degradation products 1 and 2 being 1.70 and 3.15% at zero time and 3.84 and 9.85% after 3 days, respectively.

At room temperature a variation of the *S,S*-/*R,S*-SAM diastereoisomeric ratio also occurred, being 1.63 at zero time and 1.60 and 1.45 after 2 and 3 days, respectively, probably related to a diastereoisomeric interconvertion or a different diastereoisomers rate of degradation.

It is furthermore interesting to observe that in solution II, that was frozen/thawed several times, the diastereoisomeric ratio did not change significantly (data not shown) and only a very slight SAM degradation was observed.

3.2. Study of SAM stability at 38 °C

Moreover the stability of SAM aqueous solutions was studied at 38 °C for 14 days simulating the experimental conditions used in the experiments “in vivo” studying the SAM metabolism in relation to the prevention and therapy of Alzheimer’s disease. The solution was analysed at zero time (initial solution) and after 7 and 14 days of incubation by CE in long-end injection mode. Fig. 4 shows the relative electropherograms. In these analyses it was possible to quantify both the diastereoisomeric ratio of SAM and the remarkable formation of degradation products.

Under these experimental conditions the CE technique provided a peak efficiency in the range 172,520-311,439 number of theoretical plates per meter (N/m) for *S,S*-, *R,S*-SAM, IS, products 1 and 2.

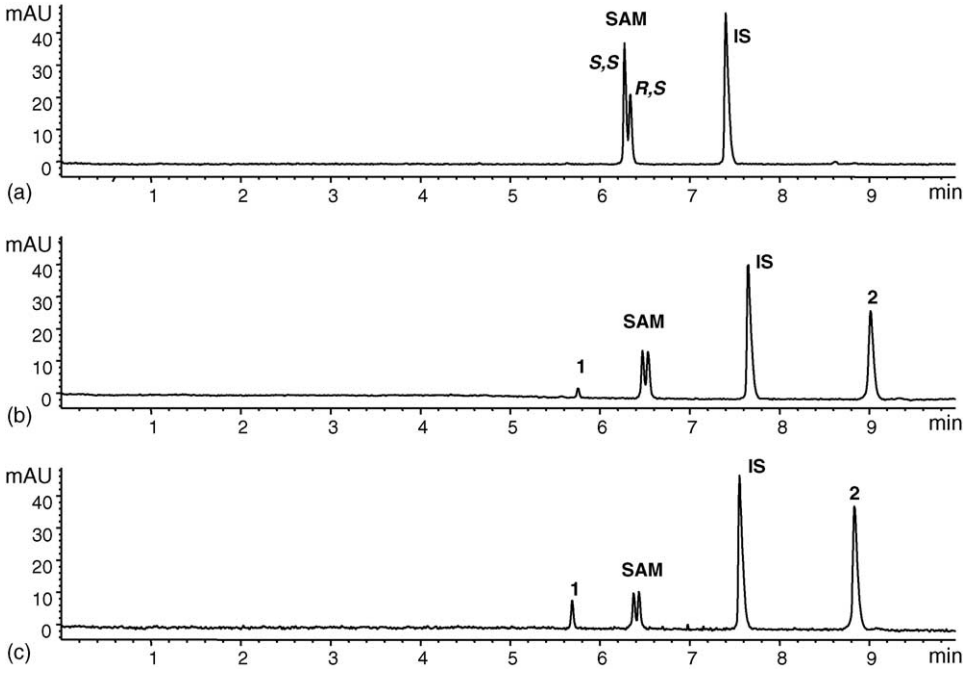

Fig. 4. Analysis of SAM aqueous solution incubated at 38 °C at (a) zero time, (b) after 7 days, (c) after 14 days by long-end injection capillary electrophoresis. For the experimental conditions see Fig. 2 and the text. IS, internal standard; 1, 2 degradation products.

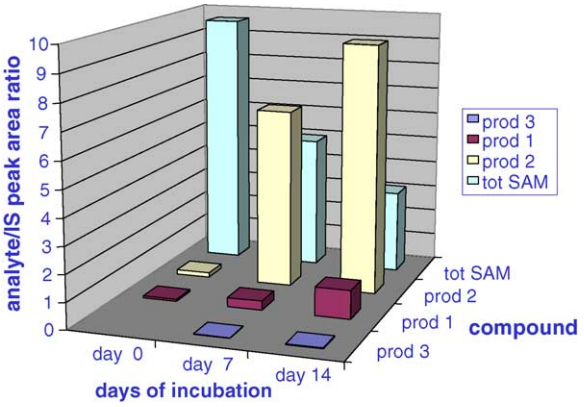

Fig. 5. Dependence of the analyte/IS peak area ratio for SAM, peak 1, peak 2 and peak 3 degradation products on the time of incubation at 38 °C. See Fig. 2 for the experimental conditions.

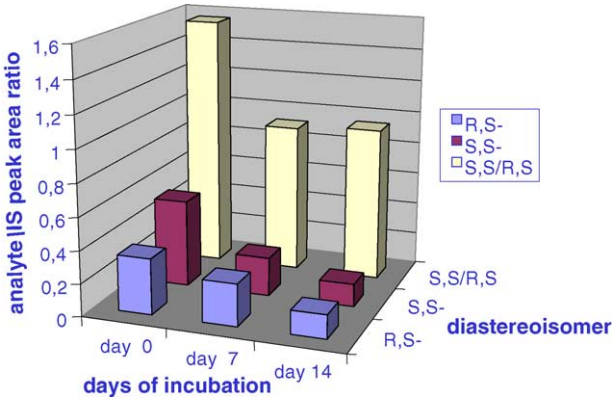

Fig. 6. Dependence of the analyte/IS peak ratio value for *S,S*- and *R,S*-diastereoisomeric forms and their ratio during the 14 days of incubation at 38 °C. See Fig. 2 for the experimental conditions.

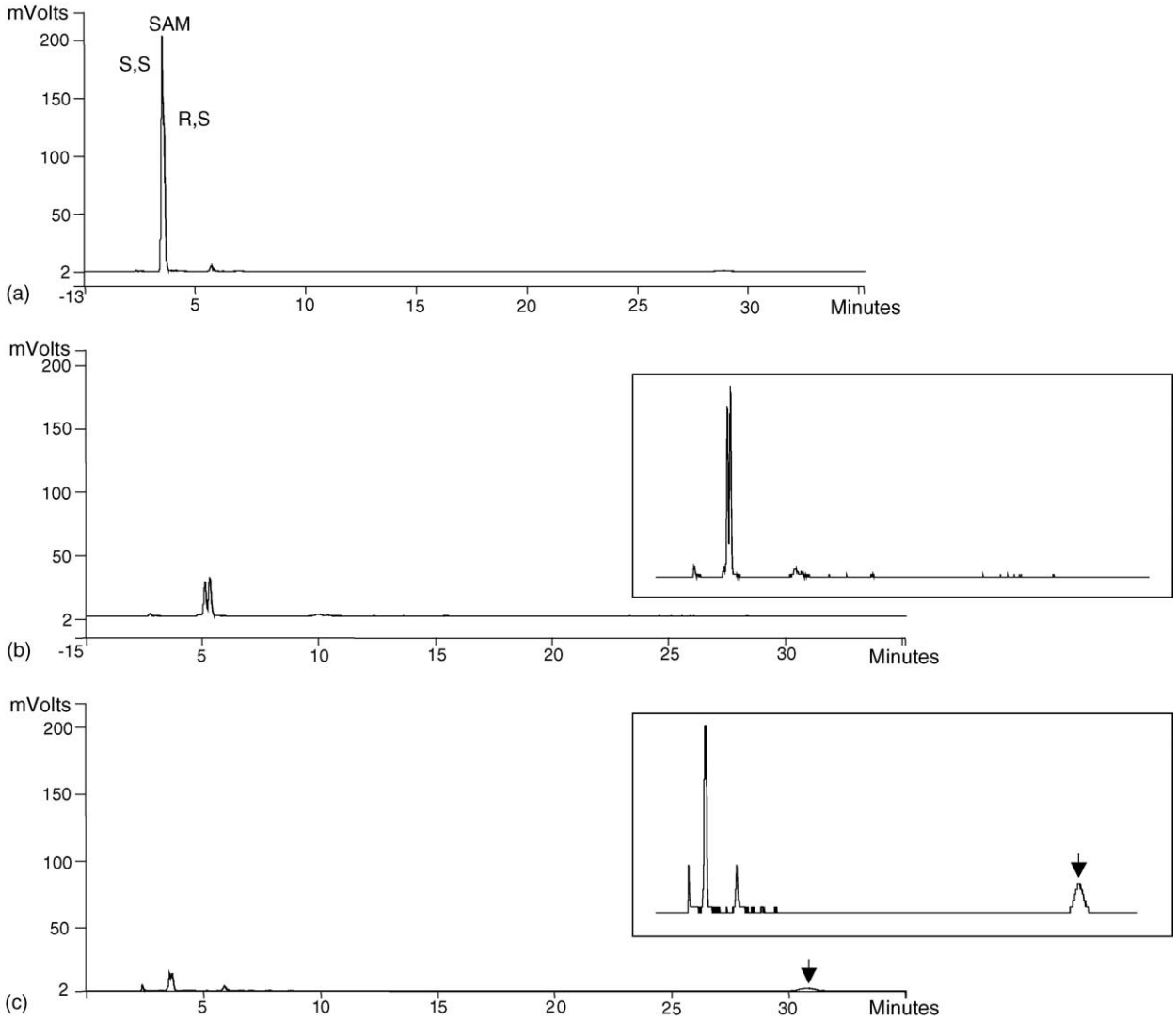

Fig. 7. HPLC analysis of SAM aqueous solution incubated at 38 °C at (a) zero time and after (b) 7 days, (c) 14 days. For a better view the *y*-axis expanded chromatograms for panels (b and c) were also added. See the text for the experimental conditions.

As it can be observed in Fig. 4a, the SAM solution at zero time contained the 39% (relative area % value) of the unnatural *R*,*S*-diastereoisomer. After 7 and 14 days of incubation the formation of two main degradation products, namely peak 1 and 2 in Fig. 4b and c, was evident. In addition, after 14 days a further degradation product, namely peak 3, a small peak visible only when injecting the concentrated samples, appeared and was quantified. Fig. 5 shows the dependence of SAM and degradation products analyte/IS peak area ratio on the time of incubation at 38 °C. The SAM content showed a decrease at increasing days of incubation and only the 51.7 and the 32.3% of the initial concentration was quantified after 7 and 14 days, respectively. Both the diastereoisomers of SAM followed the same behaviour but interesting results were observed for the diastereoisomeric ratio. In fact, after 7 and 14 days the relative area % values of *S*,*S*- and *R*,*S*-diastereoisomers changed from the initial value of 61:39 (% values) to an almost stable value, close to the racemic one. Particularly, by observing the data in Fig. 6, the *R*,*S*-/IS area ratio were 75 and 42% of the initial value after 7 and 14 days, whereas for *S*,*S*- they were 44 and 26%, respectively. Therefore it seemed that the *S*,*S*-diastereoisomer showed a stronger degradation than the *R*,*S*-form, however the occurrence of diastereoisomeric interconvertion phenomena could not be excluded.

3.3. Comparison of HPLC and CE performances for SAM stability studies

The stability of SAM aqueous solutions at 38 °C was also studied by HPLC technique: the samples were analysed at zero time, and after 7 and 14 days of incubation, as for CE analysis. The samples were injected onto a reverse phase column (HIRPB-250A, length 250 mm, ID 4.6 mm, 5 μm) and a gradient elution system was used; mobile phases were (A) 0.05 M KH_2PO_4, pH 3.5 and (B) acetonitrile (see the experimental section for details). The run time was 50 min and we observed a variation of the *S*,*S*-/*R*,*S*-SAM diastereoisomeric ratio from 1.7 at zero time to 0.9 at 14 days, and a decrement of the total content of SAM (Fig. 7). However the HPLC methodology allowed a very slow monitoring process with respect to CE and, unlike CE, it was not possible to baseline separate the diastereoisomers and to simultaneously quantify the degradation products. For these reasons, we think that CE was more effective than HPLC for a fast monitoring of the stability of SAM.

In trying to characterize the degradation products formed when the SAM solution was incubated at 38 °C, the solution sampled at day 7 was injected into an ESI–MS spectrometer by using the method of direct flow injection in positive polarity mode. On the basis of the relative abundance, together with the molecular ion of SAM (399.4), two main molecular ions were present in the solution, namely 298.5 and 102.3, suspected for the protonated S-methylthioadenosine and homoserine lactone compounds produced from the cleavage of SAM molecule.

4. Concluding remarks

CE demonstrated to be an effective analytical technique to be used for a fast monitoring of the stability of SAM solutions under different experimental conditions used for “in vitro” and “in vivo” experiments. The use of 64.5 cm long capillary of 50 μm ID and a 150 mM sodium phosphate buffer pH 2.5 allowed the simultaneous separation of SAM diastereoisomeric forms and their degradation products in less than 10 min with efficiencies values in the range 172,520-311,439 N/m.

In conclusion SAM solutions should be stored frozen to preserve the initial SAM concentration in the solution and to prevent degradation and variation of the diastereoisomeric ratio. However, although SAM degradation and variation of *S*,*S*-/*R*,*S*-ratio was observed when the solution was kept at room temperature, the SAM concentration decreased only of 5%. Several freezing/thawing cycles did not significantly affect the initial solution composition.

The stability studies of SAM at 38 °C during 14 days showed a relatively strong degradation of SAM with formation of two main products and a minor third one that strongly increased with time. The initial concentration of SAM was negatively affected by the temperature showing the need of therapy dosage adjustment to really assess the “in vivo” studies and the best way for drug administration.

In fact, after 7 and 14 days only the 52 and 32% of the initial total SAM concentration were respectively available. Moreover, after 14 days the *S*,*S*-biologically active diastereoisomer decreased of the 74%.

The ESI–MS flow injection analysis allowed the identification of suspected *S*-methylthioadenosine and homoserine lactone cleavage degradation products.

Further experiments are in progress to better identify the formation of degradation products by coupling the ESI-MS with the HPLC analytical technique.

References

[1] S.C. Lu, Int. J. Biochem. Cell Biol. 32 (2000) 391–395.
[2] S.E. Miner, J. Evrovsky, D.E. Cole, Clin. Biochem. 30 (3) (1997) 189–201.
[3] W. Herrmann, Clin. Chem. Lab. Med. 39 (8) (2001) 666–674.
[4] E. Joosten, Clin. Chem. Lab. Med. 39 (8) (2001) 717–720.
[5] M.A. Medina, J.L. Urdiales, M.I. Amores-Sanchez, Eur. J. Biochem. 268 (2001) 3871–3882.
[6] L.M. Cannon, F.N. Butler, W. Wan, Z.S. Zhou, Anal. Biochem. 308 (2002) 358–363.
[7] L.K. Revell, D.A. d’Avogno, J.C. Reepmeyer, R.C. Zerfing, J. AOAC Int. 78 (2) (1995) 353–358.
[8] S.-E. Wu, W.P. Huskey, R. Borchardt, R.L. Schowen, Biochemistry 22 (1983) 2828–2832.
[9] J.R. Matos, C.-H. Wong, Bioorg. Chem. 15 (1987) 71–80.
[10] A. Fiecchi, Double salts of *S*-adenosylmethionine, US Patent 3,954,726, (4 May 1976).
[11] G. Barbato, R. Calabria, M. Cartenì-Farina, G. D’Auria, M. De Rosa, R. Sartorio, S. Wurzburger, V. Zappia, Biochim. Biophys. Acta 991 (1989) 324–329.

[12] A. Morana, I. Di Lernia, M. Cartenì, R. De Rosa, M. De Rosa, Int. J. Pharm. 194 (2000) 61–68.
[13] A. Morana, P. Stiusi, G. Colonna, M. Lamberti, M. Cartenì, M. De Rosa, Biochim. Biophys. Acta 1573 (2002) 105–108.
[14] J.L. Hoffman, Biochemistry 25 (15) (1986) 4444–4449.
[15] W. Wang, P.M. Kramer, S. Yang, M.A. Pereira, L. Taol, J. Chromatogr. B: Biomed. Sci. Appl. 762 (1) (2001) 59–65.
[16] W. Fu, N.P. Dudman, M.A. Perry, K. Young, X.L. Wang, Biochem. Biophys. Res. Commun. 271 (1) (2000) 47–53.
[17] U. Delabar, D. Kloor, G. Luippold, B. Muhlbauer, J. Chromatogr. B: Biomed. Sci. Appl. 724 (2) (1999) 231–2388.
[18] C.K. Wise, C.A. Cooney, S.F. Ali, L.A. Poirier, J. Chromatogr. B: Biomed. Sci. Appl. 696 (1) (1997) 145–152.
[19] K.C. Panak, S.A. Giorgieri, L.E. Diaz, O.A. Ruiz, Electrophoresis 18 (1997) 2047–2049.
[20] E.O. Uthus, Electrophoresis 24 (2003) 1221–1226.
[21] E. Sbrana, E. Bramanti, M.C. Spinetti, G. Raspi, Electrophoresis 25 (2004) 1518–1521.
[22] A. Sitton, M.G. Schmid, G. Gübitz, H.Y. Aboul-Enein, J. Liq. Chromatogr. Rel. Technol. 26 (15) (2003) 2481–2490.
[23] S. Scarpa, A. Fuso, F. D'Anselmi, R.A. Cavallaro, FEBS Lett. 541 (1–3) (2003) 145–148.
[24] A. Fuso, R.A. Cavallaro, L. Orrù, F.R. Buttarelli, S. Scarpa, FEBS Lett. 508 (3) (2001) 337–340.
[25] A. Fuso, L. Seminara, R.A. Cavallaro, F. D'Anselmi, S. Scarpa, Mol. Cell. Neurosci. 28 (1) (2005) 195–204.