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Short communication

Capillary gas chromatographic determination of spermidine in diet integrators☆

V. Gambaro*, E. Casagni, L. Dell'Acqua, M. Valenti, G.L. Visconti

Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Milano, Viale Abruzzi 42, 20131 Milan, Italy

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Abstract

Biogenic polyamines, among which is spermidine (SPD, NH_2–$(CH_2)_4$–NH–$(CH_2)_3$–NH_2), are ubiquitous polycationic molecules that have a definitive role in many biological processes, such as nucleic acid metabolism, protein synthesis and cell growth. SPD is present in diet integrators because it seems to favour the hair growth. This work describes a capillary gas chromatographic (CGC) method for the quantitative determination of SPD in diet integrators using cadaverine internal standard (IS), a methyl siliconic capillary column and flame-ionization detector (FID). Diet tablets, containing SPD, are pulverized; an aliquot of powder is treated with an alkaline aqueous solution and added with IS. The suspension is extracted with diethyl ether containing ethyl chloroformate (ECF). The ether extracts, evaporated to dryness and reconstituted in ethyl acetate were analyzed in CGC/FID. Derivatives of polyamines with ECF were characterized in CGC/MS too. Validation has considered specificity, linearity, precision and accuracy of analytical method; this parameters are valid for the quantitative determination of SPD in diet integrators.

Keywords: Spermidine; Polyamines; CGC; Ethyl chloroformate (ECF); Diet integrators

1. Introduction

Spermidine (SPD, NH_2–$(CH_2)_4$–NH–$(CH_2)_3$–NH_2), a polyamine widely distributed in nature, plays a definitive role in many biological processes. Many studies proved the function of polyamines as growth factors, antioxidants, stabilizers of DNA, RNA and membranes, metabolic regulators and second messengers [1,2].

High levels of polyamines were found in many diseases such as cancer, infections, psoriasis, polycythemia rubra vera, systemic lupus erythematous, uremia, cystic fibrosis, insulin-dependent diabetes mellitus, muscular dystrophy and Alzheimer's disease.

Endogenous polyamines derive from biosynthesis and retroconversion, exogenous sources are diet and synthesis by bacteria in the gastrointestinal tract [3]. The average daily polyamine consumption in adults amounts to 350–500 μmol per person.

Many foods (cheese, beer, potatoes and green vegetables) are particularly rich in polyamines and

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* Corresponding author. Tel.: +39-02-5031-7535; fax: +39-02-5031-7571.
E-mail address: veniero.gambaro@unimi.it (V. Gambaro).

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contribute to the whole body polyamine content [4,5], besides, mineral vitaminic integrator containing SPD, advisable to fight free radicals in excess and to favour hair growth, are recently traded.

A great number of chromatographic methods for the quantitative determination of SPD are reported: by high-performance liquid chromatography (HPLC), using a pre- and post-column derivatization or different derivatizing reagents according to spectrophotometric, spectrofluorimetric or electrochemical detection; by capillary gas chromatography (CGC), involving ethyloxycarbonyl derivatives or isobutyloxycarbonyl, pentafluoropropionyl, trifluoroacetyl and heptafluorobutionyl derivatives, obtained from reaction of polyamines with appropriate acid anhydrides [6–12].

The aim of this work is the quantitative determination of SPD in diet integrators. The proposal capillary gas chromatographic method, resulting simple, fast, specific, linear, precise and accurate, is particularly suitable for resolve extractive troubles of SPD due to the complexity of a matrix like a diet integrator.

2. Experimental

2.1. Materials and reagents

Spermidine trihydrochloride and 1,5-diaminopentane dihydrochloride (cadaverine dihydrochloride, internal standard (IS)) were purchased from Sigma–Aldrich (Steinhen, Germany); ethyl acetate and ethyl chloroformate (ECF) were obtained from Merck (D-6100 Darmstadt, FR, Germany); diethyl ether from Carlo Erba Reagenti (Rodano, Italy) and NaOH from J.T. Baker (Deventer, Holland) were purchased.

Water was obtained from Milli-Q ultra-purifying system, 18.2 MΩ/cm (Millipore SA-67120 Molscheim, France). All solvents and reagents used in this study were of analytical grade.

Diet integrator batchs (Bioscalin® with Biogenina®), tablets containing SPD, and diet integrator placebo were provided by the Giuliani S.p.A. (Milan, Italy).

2.2. Apparatus

The CGC analysis were performed using a ThermoQuest (TQ) 8000 Top gas chromatograph (Thermo Quest CE Instruments, Austin) with PTV injector equipped with an AS 2000 (TQ) automatic sampler, electronic pressure control and flame-ionization detector (FID).

2.3. Chromatographic conditions

The column used was an HP-5 (5% phenyl methyl siloxane) fused-silica capillary column (12 m × 0.32 mm i.d., 0.25 μm film thickness), which was obtained from Agilent Technologies (Palo Alto, CA). Purified helium was used as carrier gas at constant pressure to assure a column flow rate of 1 ml/min. Detector gas flow rate was 9 and 350 ml/min for hydrogen and air, respectively. The column temperature was programmed from an initial 90–100 °C at a rate of 5 °C/min, increased at 20 °C/min to a final temperature of 300 °C and constant at 300 °C for 3 min. Injector initial temperature was 250 °C for 120 s and increased at 20 °C/s to 300 °C; detector temperature was 300 °C. One microlitre of the samples was injected using a split rate of 15:1. In these chromatographic conditions, the SPD and IS retention time (t_R) was about 9.4 and 6.5 min, respectively.

2.4. SPD standard solution

To prepare a standard solution (1 mg/ml), 50 mg of SPD, accurately weighed, were placed into a 50 ml volumetric flask and dissolved in water. Three standard solutions were prepared and for each one appropriate aliquots were added to the placebo obtaining standard samples.

2.5. IS solution

IS solution was prepared dissolving 20 mg of cadaverine dihydrochloride, accurately weight, in 100 ml volumetric flask; water was used as the diluent (0.2 mg/ml). Half millilitre of this solution were added to the unknown samples as well as to the standard samples for the quantitative determination.

2.6. Standard samples

In a Sovirel tube, 500 mg of placebo were spiked with appropriate amounts of SPD standard solution and volumes of water so to add total 0.5 ml and to

Table 1
SPD concentration in standard samples

n	Aliquot (ml) of SPD standard solution added to standard samples	Volume (ml) of water added to standard samples	SPD concentration in standard samples ($mg_{SPD}/500\,mg_{placebo}$)
1	0.20	0.30	0.20
2	0.30	0.20	0.30
3	0.35	0.15	0.35
4	0.40	0.10	0.40
5	0.45	0.05	0.45
6	0.50	0.00	0.50

obtain standard samples at SPD concentrations reported in Table 1. Three sets of standard samples, for each one SPD standard solution, were prepared.

2.7. Sample preparation

Ten diet integrator tablets were pulverized. To 500 mg of powder, 0.5 ml of IS solution and 10 ml of 4N NaOH were added, obtaining an homogeneous suspension.

Samples, diluted with 4 ml of diethyl ether and derivatized by adding 0.1 ml of ECF, were extracted. The organic layer was transferred to another tube and it was evaporated to dryness.

The residue was dissolved in 250 μl of ethyl acetate and an aliquot of 1 μl was injected into the gas chromatograph. Standard samples were subjected to same preparation described for the unknown samples.

3. Results and discussion

The average weight of diet integrator tablets was about 750 mg, containing 0.50 mg of SPD, therefore the method was validated analyzing the samples (500 mg of powder) with a theoretical SPD content of 0.33 mg. SPD concentration in samples was calculated by following ratio:

$$\mathrm{SPD}\left(\frac{\mathrm{mg_{SPD}}}{500\,\mathrm{mg}}\right) = \frac{A_{\mathrm{SPD}}/A_{\mathrm{IS}}}{\mathrm{RR_{mean}}}$$

where SPD ($mg_{SPD}/500\,mg$) is the SPD concentration in 500 mg of powder, A_{SPD} the SPD peak area, A_{IS} the

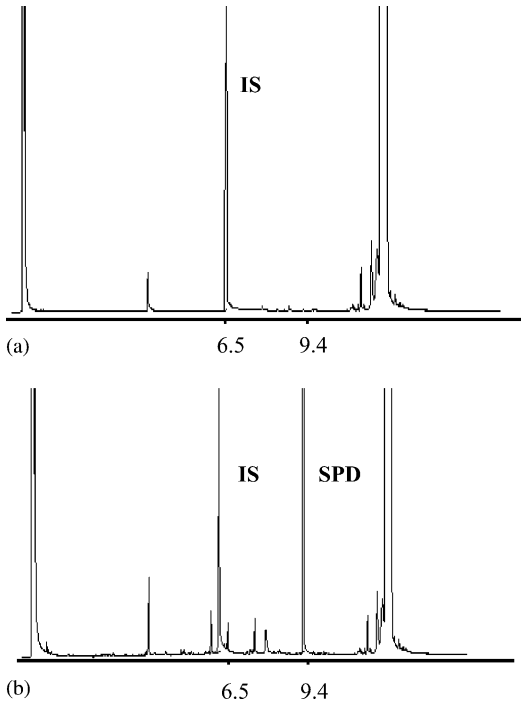

Fig. 1. CGC chromatogram of diet integrator placebo spiked with: (a) IS; (b) diet integrator sample.

Table 2
Regression analysis of the SPD determination

$mg_{SPD}/500\,mg_{placebo}$	A_{SPD}/A_{IS}	RR
Calibration curve I		
0.20	1.1938	5.97
0.30	1.7919	5.97
0.35	2.3082	6.59
0.40	2.7619	6.90
0.45	3.0625	6.81
0.50	3.5690	7.14
Calibration curve II		
0.20	1.2432	6.22
0.30	1.7996	6.00
0.35	2.2423	6.41
0.40	2.6072	6.52
0.45	2.8182	6.26
0.50	3.0345	6.07
Calibration curve III		
0.20	1.4908	7.45
0.30	2.1352	7.12
0.35	2.1993	6.28
0.40	2.5484	6.37
0.45	2.8945	6.43
0.50	3.1121	6.22

IS peak area, and RR_{mean} the mean of RR calculated in linearity study:

$$RR = \frac{A_{SPD}/A_{IS}}{C_{st}}$$

where C_{st} the SPD concentration (mg) in standard samples.

The performance of this gas chromatographic method was tested evaluating specificity, linearity, precision and accuracy.

3.1. Specificity

No interference at the retention time of the substances (SPD or IS) was obtained in the analyses of placebo and placebo spiked with SPD and IS (Fig. 1).

3.2. Linearity

The linearity was evaluated on three sets of standard samples in the range 0.20–0.50 mg_{SPD}/500 $mg_{placebo}$, preparing one calibration curve for each set by the least squares method. Linear regression lines were obtained by plotting the peak area (ratios of SPD peak area to IS peak area) versus the SPD concentration in each standard sample (Tables 2 and 3).

Table 3
Statistical results of linearity

R^2	Intercept	Slope	RR_{mean}	CV%
Calibration curve I				
0.9961	−0.4828	7.9927	6.5642	7.5
Calibration curve II				
0.9937	0.0165	6.2026	6.2451	3.2
Calibration curve III				
0.9929	0.4253	5.3765	6.6471	7.7

3.3. Precision

The intra-day precision was evaluated by RR_{mean} and coefficient of variation (CV%) of six standard samples containing 0.35 mg_{SPD}/500 $mg_{placebo}$ analyzed in the same day. The inter-day precision was determined analyzing in three different days six standard samples ($n = 18$), at 0.20, 0.30, 0.35, 0.40, 0.45, and 0.50 mg of SPD in 500 mg of placebo, and calculating RR_{mean} and CV% (Table 4).

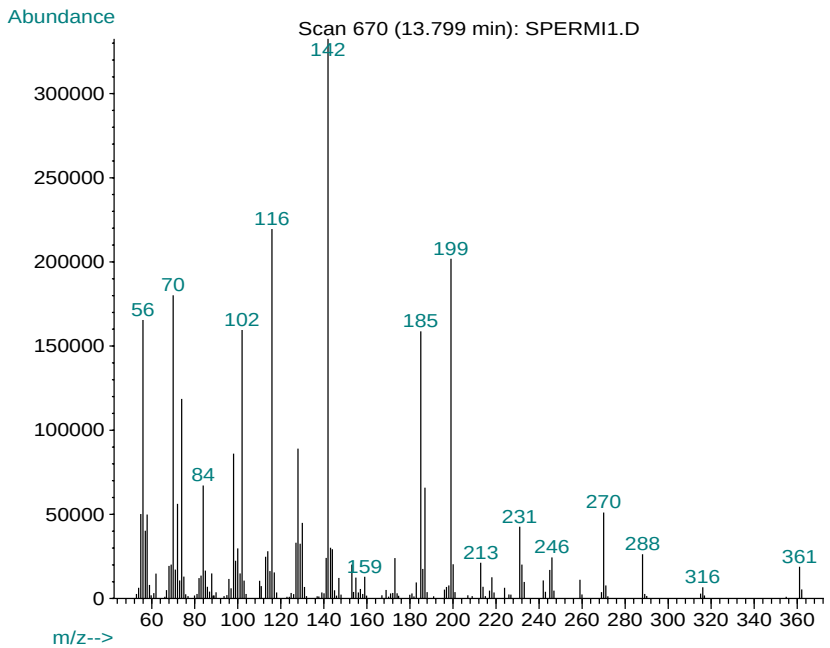

Fig. 2. Mass spectrum of SPD derivative with ECF.

Table 4
Precision

n	RR_{mean}	S.D.	CV%
Intra-day precision			
6	6.1283	0.009	2.8
Inter-day precision			
18	6.4855	0.437	6.7

Table 5
Results from CGC analysis of SPD in three diet integrator batches

n	$mg_{SPD}/500\,mg_{placebo}$ (mean)	CV%
Batch 1		
5	0.309	6.37
Batch 2		
5	0.309	5.60
Batch 3		
5	0.313	6.88

3.4. Accuracy

Accuracy was calculated as recovery percentage between the found and known concentrations (Rec% = (experimental value/theoretical value) × 100). Accuracy data were obtained by analysis of the standard samples at 0.30, 0.35, and 0.40 $mg_{SPD}/500$ mg SPD concentration, for each calibration curve ($n = 9$) and the Rec%(mean) was 99.66%.

3.5. Analysis of diet integrator tablets

The results of the SPD quantitative determination by proposal method in the examined diet integrator batches are reported in Table 5. Derivatives of SPD with ECF were characterized in CGC/MS too (Fig. 2).

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