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Improved high-performance liquid chromatographic method for the determination of polyamines as their benzoylated derivatives: application to P388 cancer cells

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Abstract

A simple reversed-phase HPLC method was developed for the determination of eight polyamines or monoacetylpolyamines, as their benzoylated derivatives. Interfering products, inherent to the benzoyl chloride derivatization technique (benzoic acid, methyl benzoate and benzoic anhydride), were identified. A new derivatization procedure for their total elimination was developed without any loss of sensitivity and selectivity. Not only the HPLC method was validated, but also the choice of an internal standard was investigated. The results show that it is possible to use this HPLC assay to determine the polyamine content in P388 cancer cells. Furthermore, the method is now being used to evaluate the uptake of various polyamines by P388 cancer cells and by other cancer and parasitic cells.

1. Introduction

Polyamines are aliphatic polycations that are essential for proliferative and differentiative processes both in eukaryotic and prokaryotic cells [1,2]. Although it is accepted that they are implicated in a variety of cell functions involving DNA replication, protein synthesis and gene expression [3,4], their exact modes of action remain unknown. Therefore, the determination of polyamines is necessary in order to clarify their precise roles.

Many studies have been conducted on the HPLC separation of benzoylated polyamines with a short analysis time, but they were often related to classical polyamines: putrescine, cadaverine, spermidine and spermine. When more polyamines were separated, the method was developed for special application and no other polyamine could be added without overlapping [5–7]. Moreover, we were not able to find a published method suitable for the total separation of benzoylated putrescine, 1,3-diaminopropane and cadaverine [8,9].

In this study, an HPLC assay was developed which, for the first time, allowed the total separation of these three benzoylated derivatives, as well as many other polyamine derivatives.

During the development of the HPLC method, interfering products were found which were inherent to the benzoylation derivatization pro-

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cedure. This procedure has been studied in order to identify those impurities and to discard them before HPLC analysis. The degradation of the impurities was performed by a non-destructive process for polyamine and acetylpolyamine derivatives without any loss of sensitivity and selectivity.

This HPLC assay has proved to be suitable for the analysis of polyamines in biological media, and was used to determine the polyamine content of P388 cancer cells.

2. Experimental

2.1. Chemicals

Spermidine trihydrochloride, spermine tetrahydrochloride, 1,4-diaminobutane dihydrochloride (putrescine) were supplied by Fluka (Buchs, Switzerland); 1,3-diaminopropane, 1,5-diaminopentane (cadaverine), 1,6-diaminohexane, N^1-acetylspermidine dihydrochloride and N^1-acetylspermine trihydrochloride were provided by Sigma (St. Louis, MO, USA). Benzoyl chloride was obtained from Janssen Chimica (Geel, Belgium). Benzoic anhydride and ethyl 4-hydroxybenzoate were purchased from Aldrich (Steinheim, Germany). Methyl benzoate, benzoic acid and all other reagents were analytical-reagent grade and were supplied by Merck (Darmstadt, Germany).

2.2. Benzoic anhydride degradation study

A 2-ml aliquot of a methanol–water (55:45; v/v) solution containing 39.2 or 392 nmol/ml of benzoic anhydride was incubated at 35°C. An aliquot was withdrawn every hour for 9 h and analyzed by HPLC. Ethyl 4-hydroxybenzoate was used as an internal standard.

2.3. Polyamine derivatization procedure

Sodium hydroxide, 2 *M* (1.0 ml) and benzoyl chloride (5 μl) were added to 1 ml of a biological sample or a sample containing 2.5–250 nmol of the different standard polyamines. The mixture was briefly shaken in a vortex and, after 20 min, saturated sodium chloride solution (2.0 ml) and chloroform (2.0 ml) were added. This solution was vortex-mixed for 1 min, then centrifuged at 2000 *g* for 10 min, after which 1.5 ml of the lower organic phase was withdrawn and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.5 ml of mobile phase and left overnight (15 h) at 35°C in screw-cap glass tubes. This solution was treated with 2 *M* sodium hydroxide (0.2 ml) and chloroform (2.0 ml), then mixed for 1 min and centrifuged for 10 min at 2000 *g*; 1.5 ml of the organic phase was evaporated under a stream of nitrogen. Before injection and HPLC analysis the remaining residue was dissolved in 0.5 ml of the mobile phase.

2.4. Chromatographic analysis

Separation of benzoylated amines and interfering products was carried out with isocratic reversed-phase HPLC using a Gilson Model 305 pump (Middleton, WI, USA), a Rheodyne 7125 100-μl loop injector (Cotati, CA, USA) and an Ultrasphere C_{18} column (250 mm × 4.6 mm I.D., 5 μm particle size) from Beckman (Berkeley, CA, USA). The mobile phase was a mixture of methanol–water (55:45, v/v) delivered at a flow-rate of 1.0 ml/min. Benzoic anhydride, its related products and the amine derivatives were detected spectrophotometrically at 234 nm with a diode array detector (module 168 from Beckman). The detector output was integrated and quantified on the Gold system (Beckman).

2.5. P388 cells recovery study

A 2-ml aliquot of a suspension containing 10^7 cells/ml was deposited on 6 ml of a 4% albumin–water solution. The suspension was centrifuged at 450 *g* for 5 min and the supernatant was replaced by 1 ml of $HClO_4$ (6%). Then, the mixture was homogenized and spiked with 0.3 ml of aqueous solution containing polyamines (putrescine, 2, 4, 6, 8, 10 nmol; spermidine, 20, 40, 50, 60, 80 nmol; spermine, 10, 20, 30, 40, 60 nmol) and internal standards (cadaverine, 5

nmol; 1,6-diaminohexane, 50 nmol; 1,8-diaminooctane, 50 nmol, respectively). The suspension was vortexed and centrifuged at 450 *g* for 10 min. The supernatant was withdrawn and derivatized as described above.

The spiking concentrations were chosen to be about twice the endogenous amounts of cells which were determined from unspiked samples.

2.6. Maintenance of P388 cell culture

Cells were maintained in RPMI 1640 (Gibco, Ghent, Belgium) medium supplemented with 10% (v/v) foetal calf serum (Gibco), 2 m*M* glutamine (Gibco), 10 m*M* HEPES (Gibco) and 100 U/ml penicillin–streptomycin solution (Gibco). Cells were incubated at 37°C in an air-humidified atmosphere (5% CO_2–95% air).

3. Results and discussion

Our goal was to develop an HPLC method which allowed the total separation and the determination of eight polyamines often found in biological media, namely: N^1-acetylspermidine, putrescine, 1,3-diaminopropane, cadaverine, N^1-acetylspermine, 1,6-diaminohexane, spermidine and spermine. Moreover, this method had to be reusable for many applications. For this reason, isocratic conditions were chosen. Furthermore, the method had to suitably resolve the different polyamines in a relatively short time. Table 1 gives the different retention characteristics of the eight polyamines derivatives achieved with the three mobile-phase compositions investigated.

Good separation of all the polyamines was achieved using methanol–water (50:50), but the spermine derivative eluted very late. Moreover, N^1-acetylspermine and 1,6-diaminohexane were not totally separated. The methanol–water (60:40) mobile phase provided an analysis time of 20 min, but the separation of putrescine, diaminopropane and cadaverine was not satisfactory. Finally, methanol–water (55:45) was chosen for the remaining experiments. This mobile phase allowed the total separation of the eight polyamines in 40 min under isocratic conditions.

3.1. Interfering products study

In previous studies it was reported that with the benzoyl chloride derivatization technique of polyamines no side reaction compound was produced [10]. In our investigations, using the derivatization procedures previously reported [8,11,12], undesired products such as benzoic acid, methyl benzoate and benzoic anhydride appeared. Moreover, in the method we have developed (methanol–water, 55:45), methyl benzoate and benzoic anhydride were respectively

Table 1
Retention characteristics of polyamine derivatives

Compound	CH_3OH–H_2O (50:50, v/v)		CH_3OH–H_2O (55:45, v/v)		CH_3OH–H_2O (60:40, v/v)	
	Retention time	R_s	Retention time	R_s	Retention time	R_s
N^1-Acetylspermidine	9.3		5.9		4.7	
Putrescine	12.4	2.82	7.7	2.77	6.0	2.00
1,3-Diaminopropane	13.8	1.08	8.5	1.05	6.5	0.77
Cadaverine	15.6	1.29	9.2	1.12	6.9	0.62
N^1-Acetylspermine	24.5	6.00	11.5	2.42	7.6	1.00
1,6-diaminohexane	23.4[a]	0.81	12.6	1.10	8.7	1.38
Spermidine	36.7	6.10	16.9	3.74	10.7	2.50
Spermine	109.8	17.61	38.5	11.36	19.8	7.58

[a] 1,6-Diaminohexane eluted prior to N^1-acetylspermine with the CH_3OH–H_2O (50:50) mobile phase. Amount of each compound: 5.6 nmol per injection.

superimposed on N^1-acetylspermine and spermine derivatives. Benzoic acid eluted very early and did not interfere (Fig. 1). Interfering products were identified by their UV spectra and by comparison with retention times of standard samples.

Our findings are in accordance with the recent results of Watanabe et al. [12] who, surprisingly, were the first to detect these interfering products. However, the technique they described for eliminating the interferences by shaking benzoylation solution overnight at 60°C was unsuccessful. As expected, methyl benzoate and benzoic anhydride had totally disappeared while benzoic acid was still present in a small amount. It was not possible to recover the acetyl derivatives since partial degradation, into non-acetyl compounds, occurred due to high temperatures.

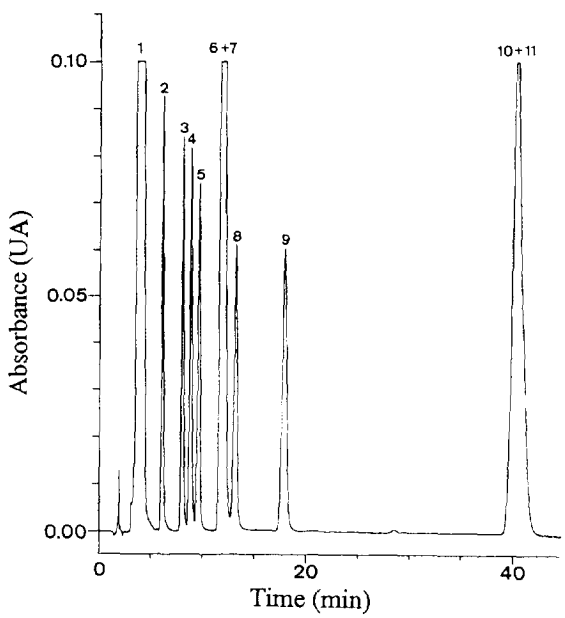

Fig. 1. HPLC chromatogram of benzoylated polyamines without a second extraction. After the first extraction with chloroform, the residue was dissolved in 0.5 ml of mobile phase. A volume of 50 μl of this solution was injected. The amount of each compound was 1.5 nmol per injection. Peaks: 1 = benzoic acid; 2 = N^1-acetylspermidine; 3 = putrescine; 4 = 1,3-diaminopropane; 5 = cadaverine; 6 = methyl benzoate; 7 = N^1-acetylspermine; 8 = 1,6-diaminohexane; 9 = spermidine; 10 = spermine; 11 = benzoic anhydride.

Moreover, the acetyl derivatives were poorly extracted by diethyl ether.

Verkoelen et al. [8] used chloroform instead of diethyl ether to extract the acetyl derivatives. As a matter of fact, they were extracted but, nevertheless, the recovery was not good. The heating had decreased the peak area by approximately two-fold.

Taibi and Schiavo [11] did not mention any interfering products. They washed the organic phase with sodium hydroxide after extraction with diethyl ether, and eliminated water with anhydrous sodium sulphate. The use of this method did not allow for the elimination of interfering products, and acetyl derivatives were not extracted. The use of chloroform also did not remove the interfering products.

So, until now, no method was able to eliminate benzoylation impurities without any degradation of acetyl derivatives or was suitable to totally and specifically recover the polyamine derivatives from the benzoylation medium.

As described by Watanabe et al. [12], benzoic chloride and benzoic acid can react together to form benzoic anhydride. Since benzoyl chloride is decomposed by water to benzoic acid and as this acid is in the sodium form, benzoic anhydride is the only interfering compound extractable by the organic phase. In the assay, it was proposed to degrade benzoylation impurities in a mixture of water and methanol. Benzoic anhydride can react with water to form two benzoic acids, or with methanol to form benzoic acid and methyl benzoate, which are the three interfering products (Fig. 2). If benzoic anhydride can be totally decomposed into its products, a second extraction with chloroform would give a solution free of interfering products (methyl benzoate was degraded before the second extraction, due to the temperature and the NaOH solution).

In order to eliminate benzoic anhydride, the kinetics of its degradation in a water–methanol mixture (the mobile phase) at 35°C was investigated. The choice of using the mobile phase as the mixture was arbitrary. The degradation of benzoic anhydride in other solvent conditions was not investigated. However, it was demon-

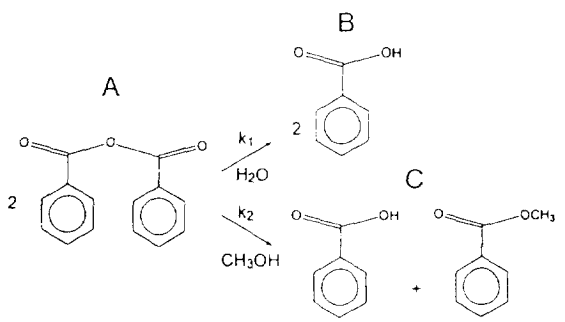

Fig. 2. Degradation pattern of benzoic anhydride in a methanol–water mixture.

strated that the procedure described is a very efficient one.

The degradation pattern of benzoic anhydride in the methanol–water mixture (Fig. 2) seemed to correspond to competitive first-order kinetics, with:

$$A_t = A_0 e^{-(k_1+k_2)t}$$

$$B_t = \frac{2A_0k_1}{k_1+k_2}(1 - e^{-(k_1+k_2)t})$$

$$C_t = \frac{2A_0k_2}{k_1+k_2}(1 - e^{-(k_1+k_2)t})$$

where A_0 and A_t are the concentrations of A (2 benzoic anhydrides) at times 0 and t, B_t is the concentration of B (2 benzoic acids) at time t, C_t is the concentration of C (1 benzoic acid + 1 methyl benzoate) at time t, k_1 and k_2 are the kinetic constants of the two degradation patterns.

A direct kinetics degradation study of benzoic anhydride was not possible. As it is not stable in water or methanol, it was impossible to express a linear relation between surfaces and concentrations. However, reaction products are stable at 35°C and it was possible to study the degradation pattern of benzoic anhydride by way of reaction products. The curve was linear for both benzoic acid and methyl benzoate between 0.6 and 900 nmol/ml.

The concentrations of benzoic anhydride initially present in the solution before the second extraction was estimated from the appearing concentrations of benzoic acid and methyl benzoate. For the degradation study, two concentrations bracketing the expected concentrations of benzoic anhydride were incubated at 35°C.

The experimental results for benzoic acid and methyl benzoate were transformed in terms of B (2 benzoic acids) and C (1 benzoic acid + 1 methyl benzoate). The latter results were adjusted to the parametric functions of competitive first-order kinetics with a software package called FADHA [13–15]. The algorithm of this software is based on the simplex method used to minimize a non-linear cost function. Parameters were k_1, k_2 and A_0. Fig. 3 represents the degradation pattern of A (benzoic anhydride) and the appearance patterns of B (2 benzoic acids) and C (1 benzoic acid + 1 methyl benzoate). The results in Table 2 show that benzoic anhydride (in the studied range of concentrations) lowered itself at 35°C according to a first-order kinetics reaction as k was independent of the concentration. This meant that overnight (15 h), only 0.02% of benzoic anhydride remained in the solution before the second extraction.

As expected, the derivatization method of polyamines, as described in the experimental part, allowed the total elimination of the interfering products without loss of efficiency. Chromatograms of polyamines and their acetyl derivatives are shown in Fig. 1 (without any second

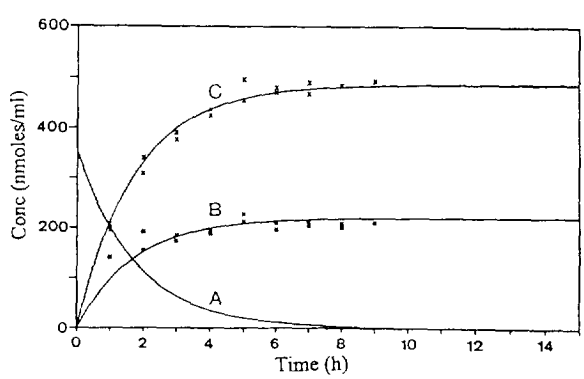

Fig. 3. Kinetics of benzoic anhydride degradation. An aliquot (2 ml) of benzoic anhydride (392 nmol/ml) in the mobile phase was incubated at 35°C; 50 μl of this solution were injected every hour during 9 h.

Table 2
Calculated parameters k_1, k_2, K, $t_{1/2}$ and A_0 according to competitive first-order degradation kinetics of benzoic anhydride

Concentration (nmol/ml)	k_1 (mean ± S.D.) (h^{-1})	k_2 (mean ± S.D.) (h^{-1})	K ($k_1 + k_2$) (mean ± S.D.) (h^{-1})	$t_{1/2}$ (mean ± S.D.) (h)	A_0 (mean ± S.D.) (nmol/ml)
39.2	0.180 ± 0.005	0.410 ± 0.008	0.590 ± 0.013	1.18 ± 0.02	43 ± 1
392	0.180 ± 0.010	0.390 ± 0.020	0.570 ± 0.030	1.22 ± 0.02	356 ± 4

extraction) and in Fig. 4 (with a second extraction).

3.2. Standard curves for polyamines derivatized in aqueous solutions

Tables 3 and 4 show that the calibration curves exhibited excellent linearity for all the polyamines. The correlation coefficients were greater than 0.9988 for the concentration range investigated (5–500 nmol).

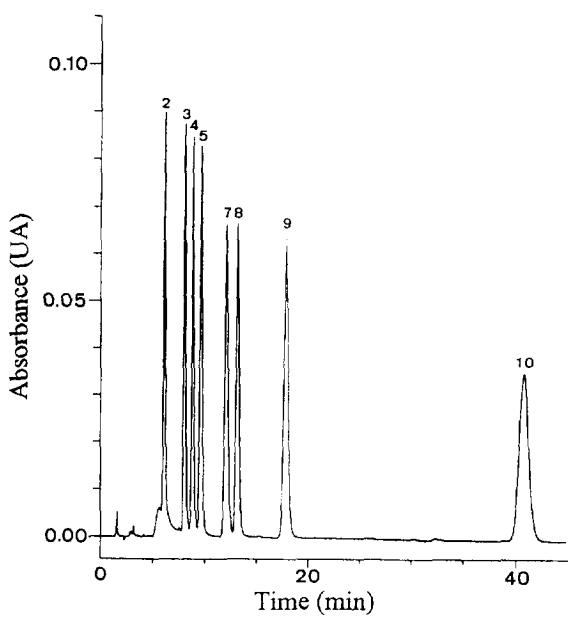

Fig. 4. HPLC chromatogram of benzoylated polyamines with a second extraction, as described in the experimental part. The amount of each compound was 1.5 nmol per injection. Peaks: 2 = N^1-acetylspermidine; 3 = putrescine; 4 = 1,3-diaminopropane; 5 = cadaverine; 7 = N^1-acetylspermine; 8 = 1,6-diaminohexane; 9 = spermidine; 10 = spermine.

The values of the slopes (a) and the intercepts (b) collected in Table 3 show that the relative standard deviations of the slopes were around 10% for all compounds. The intercept values for the standard curves for all polyamines ranged from −0.01 to −0.11 and were not significantly ($P < 0.05$) different from zero (except for cadaverine).

3.3. Precision of the derivatization procedure and HPLC assay

The mean intra- and inter-assay relative standard deviations varied, respectively, from 1.3 to 3.0% and from 6.0 to 10.0%. The 1,6-diaminohexane was used as the internal standard (I.S.) for HPLC, to compensate for derivatization, injection and other manipulation variations. The results indicated that this compound can function as an internal standard with good precision.

Table 4 demonstrates that the inter-assay variations (C.V., %) were often concentration-independent.

3.4. Sensitivity

The limits of detection (LOD) and the limits of quantification (LOQ) were used to assess the sensitivity of the method. The LOD and the LOQ were defined as the amount of analyte giving a peak height three and ten times, respectively, the maximum noise peak height of a blank biological sample observed at the retention time of each analyte. The LOD was between 2 and 12.5 pmol and the LOQ between 16.5 and 40 pmol. For putrescine, spermidine and sper-

Table 3
Linear regression parameters and intra-day and inter-day mean coefficients of variation for HPLC assay of polyamine and monoacetyl polyamine derivatives ($n = 4$)

Compound	Slope (mean ± S.D.)	Intercept (mean ± S.D.)	Correlation coefficient (mean ± S.D.)	Inter-day[a] mean C.V. (%)	Intra-day[a] mean C.V. (%)
N^1-Acetylspermidine	0.012 ± 0.001	−0.02 ± 0.02	0.9992 ± 0.0008	9.5	1.6
Putrescine	0.019 ± 0.002	−0.06 ± 0.08	0.9989 ± 0.0007	8.8	1.4
1,3-Diaminopropane	0.016 ± 0.001	−0.03 ± 0.04	0.9988 ± 0.0008	10.0	2.2
Cadaverine	0.023 ± 0.002	−0.11 ± 0.05	0.9993 ± 0.0003	7.2	1.6
N^1-Acetylspermine	0.014 ± 0.002	−0.01 ± 0.06	0.9995 ± 0.0002	6.6	1.3
Spermidine	0.022 ± 0.003	−0.04 ± 0.05	0.9996 ± 0.0003	8.5	1.5
Spermine	0.027 ± 0.005	−0.05 ± 0.01	0.9995 ± 0.0002	6.0	3.0

[a] Mean coefficients of variation are expressed with regard to the internal standard: 1,6-diaminohexane. Intra-day and inter-day C.V. for 1,6-diaminohexane were 2.5% and 8.5% ($n = 10$). Intra-day mean C.V. are calculated from six different concentrations of a standard polyamine mixture. For each concentration, four samples were analysed. Inter-day mean C.V. are calculated from six different concentrations of a standard polyamine mixture. For each concentration, a different sample was analysed on four different days.

mine, these limits were unchanged after extraction from P388 cells.

3.5. Standard curves and relative recoveries for endogenous polyamines extracted from P388 cells

Three endogenous polyamines were identified and determined in P388 cells: putrescine, spermidine and spermine (Fig. 5 and Table 5). These compounds had very different chromatographic behaviours. So, the linearity of the method, the recoveries and the precision of the extraction were estimated from peak area ratios of the polyamine to an I.S. which had a retention time close to the compound of interest. These parameters were considered to discriminate between the different internal standards. The results (Table 6) indicated that diaminohexane can function as an I.S. for the three polyamines. Cadaverine seemed to be a more appropriate I.S. to determine putrescine, but the recovery values were not statistically ($P < 0.05$) different from those related to 1,6-diaminohexane. The mean recovery for spermine was the lowest and significantly ($P < 0.05$) different from 100%; however, both the precision and linearity were very good for this compound. This finding could be explained by matrix interactions of spermine which has numerous cationic functions, or by

Table 4
Influence of the concentration on the inter-assay peak area ratio variation ($n = 4$)

Compound	Absorbance (mean ± S.D.)					
	5 nmol	10 nmol	20 nmol	50 nmol	100 nmol	500 nmol
N^1-Acetylspermidine	0.073 ± 0.005	0.16 ± 0.01	0.26 ± 0.03	0.62 ± 0.07	1.0 ± 0.1	6.2 ± 0.7
Putrescine	0.12 ± 0.01	0.22 ± 0.02	0.36 ± 0.02	0.9 ± 0.1	1.6 ± 0.1	9.6 ± 0.9
1,3-Diaminopropane	0.09 ± 0.01	0.20 ± 0.02	0.30 ± 0.03	0.72 ± 0.05	1.3 ± 0.1	7.7 ± 0.7
Cadaverine	0.11 ± 0.01	0.22 ± 0.02	0.42 ± 0.02	0.99 ± 0.03	1.9 ± 0.1	1.5 ± 0.9
N^1-Acetylspermine	0.070 ± 0.007	0.14 ± 0.02	0.265 ± 0.008	0.64 ± 0.03	1.23 ± 0.09	7.54 ± 0.09
Spermidine	0.12 ± 0.02	0.25 ± 0.02	0.46 ± 0.05	1.13 ± 0.09	2.0 ± 0.2	11.9 ± 0.7
Spermine	0.14 ± 0.01	0.28 ± 0.03	0.52 ± 0.01	1.3 ± 0.1	2.4 ± 0.2	14.8 ± 0.6

Table 5
Basal amounts of polyamines in P388 cancer cells ($n = 6$)

Compound	Concentration (mean ± S.D.) (nmol/10^6 cells)
Putrescine	0.07 ± 0.02
Spermidine	1.1 ± 0.3
Spermine	0.4 ± 0.1

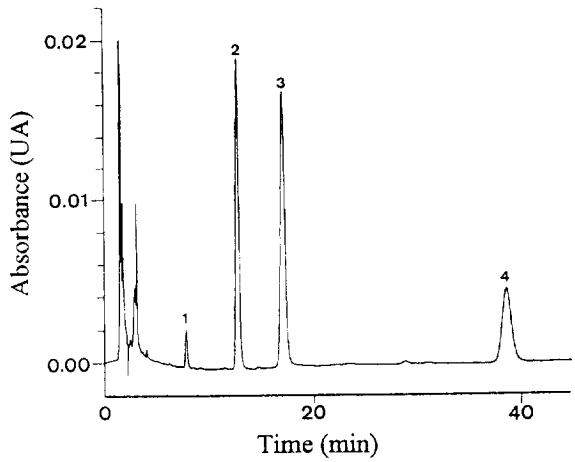

Fig. 5. Chromatogram of benzoylated polyamines from P388 cancer cells (basal level). Peaks: 1 = putrescine; 2 = 1,6-diaminohexane; 3 = spermidine; 4 = spermine.

behavioural differences between spermine and the I.S. during the assay. However, the differences between mean recovery values of spermine either related to 1,6-diaminohexane or to 1,8-diaminooctane ($t_r = 29$ min) were not statistically significant ($P < 0.05$). So, the use of an I.S. more structurally similar to spermine (1,8-diaminooctane) did not improve the estimation of the relative recovery of spermine.

Table 6 also indicates that relative recoveries and relative standard deviations were concentration-independent over the range tested. Moreover, comparison of Tables 3 and 6 showed that the precision of the HPLC assay was not influenced by the extraction step from biological media and that the variability was mainly caused by the derivatization technique.

Table 6
Influence of the internal standard on the mean relative recoveries, and linearity of polyamines extracted from P388 cancer cells ($n = 4$)

Compound	Spiked concentration (nmol)	I.S.	Relative recovery (mean ± S.D.) (%)	Mean relative recovery (mean ± S.D.) (%)	Slope (mean ± S.D.)	Intercept (mean ± S.D.)	Correlation coefficient (mean ± S.D.)
Putrescine	2	Cadaverine	108 ± 10	104 ± 5	0.139 ± 0.005	0.01 ± 0.05	0.999 ± 0.001
	4		105 ± 8				
	6		103 ± 6				
	8		100 ± 9				
	10		102 ± 6				
		Diaminohexane		101 ± 6	0.0119 ± 0.0009	−0.001 ± 0.005	0.996 ± 0.003
Spermidine	20	Diaminohexane	95 ± 8	94 ± 7	0.0146 ± 0.0008	−0.01 ± 0.05	0.991 ± 0.007
	40		92 ± 11				
	50		90 ± 6				
	60		101 ± 10				
	80		93 ± 7				
Spermine	10	Diaminohexane	85 ± 3	87 ± 3	0.016 ± 0.002	0.02 ± 0.02	0.9995 ± 0.0007
	20		89 ± 7				
	30		85 ± 4				
	40		86 ± 2				
	60		83 ± 1				
		Diaminooctane		79 ± 7	0.015 ± 0.002	0.03 ± 0.07	0.99 ± 0.01

4. Conclusions

A sensitive and reproducible HPLC method has been developed to determine eight polyamines.

The selectivity of this method over previously described methods was considerably improved by an original clean-up of the derivatized polyamine solution. The new conditions of extraction were achieved by the comprehension of the ionization, partition and kinetic behaviour of the identified impurities generated during derivatization.

The HPLC assay was applied to the quantification of three major polyamines in P388 cancer cells, but it should be possible to use this method to determine other polyamines or polyamine metabolism products in other biological samples.

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References

[1] C.W. Tabor and H. Tabor, Microbiol Rev., 49 (1985) 81.
[2] A.E. Pegg, Biochem. J., 234 (1986) 249.
[3] H.S. Basu, B.G. Feuerstein and L.J. Marton, in J-Ph. Moulinoux and V. Quemener (Editors), Les Polyamines, Flammarion Médecine-Sciences, Paris, 1991, p. 3.
[4] A.E. Pegg and P. Mc Cann, Am. J. Physiol., 243 (1982) C212.
[5] S. Asotra, P.V. Mladenov and R.T. Burke, J. Chromatogr., 407 (1987) 227.
[6] P.J. Oefner, S. Wongyai and G.K. Bonn, Clin. Chim. Acta, 205 (1992) 11.
[7] S. Wongyai, P.J. Oefner and G.K. Bonn, J. Liq. Chromatogr., 12 (1989) 2249.
[8] C.F. Verkoelen, J.C. Romijn, F.F. Schroeder, W.P. Van Schalkwijk and T.A.W. Splinter, J. Chromatogr., 426 (1988) 41.
[9] R.D. Slocum, H.E. Flores, A.W. Galston and L.H. Weinstein, Plant Physiol., 89 (1989) 512.
[10] N. Seiler, J. Chromatogr., 379 (1986) 157.
[11] G. Taibi and M.R. Schiavo, J. Chromatogr., 614 (1993) 153.
[12] S. Watanabe, T. Saito, S. Sato, S. Nagase, S. Ueda and M. Tomita, J. Chromatogr., 518 (1990) 264.
[13] A.V. Hill, J. Physiol., 40 (1910) II.
[14] F. Abi Khalil, J. Dubois, M. Hanocq and G. Atassi, Eur. J. Drug Metab. Pharmacokinet., 11 (1986) 51.
[15] J. Dubois, F. Abi Khalil, M. Hanocq, G. Atassi and R. Arnould, J. Pharm. Belg., 44 (1989) 181.