ORIGINAL RESEARCH

Analysis of polyamines as carbamoyl derivatives in urine and serum by liquid chromatography–tandem mass spectrometry

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ABSTRACT: A quantitative analysis of polyamines in urine and serum by liquid chromatography–tandem mass spectrometry (LC-MS/MS) is described. The polyamines were carbamylated with isobutyl chloroformate, extracted with diethyl ether under pH 9.0, and analyzed by LC-MS/MS with single reaction monitoring mode. The limit of quantification was 1 ng/mL based on a signal-to-noise ratio >3, and the correlation coefficient (r^2) for the calibration curves was >0.99 for both urine and serum samples. The present method was applied to urine and serum samples from 30 breast cancer patients and 30 normal female controls. There was no significant difference in the urinary polyamine levels between breast cancer patients and controls. However, 1,3-diaminopropane, putrescine, spermine and *N*-acetylspermidine levels in serum increased in breast cancer patients. These four serum polyamines may be a good index to study both production and metabolism of polyamines, and a useful tool in assessment of the polyamine status of breast cancer patients. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: polyamine; carbamoylation; urine; serum; LC-MS/MS

INTRODUCTION

Polyamines are active biogenic amines which play an important role in cell growth and proliferation and the synthesis of proteins and nucleosides (Pegg, 1988). They are also scavengers of reactive-oxygen species, thereby protecting DNA, protein and lipids from oxidative damage (Chattopadhyay *et al.*, 2003), so they have been investigated as anti-cancer agents or tumor markers (Lee *et al.*, 1998; Parham and Robertson, 1989; Seiler *et al.*, 1981; Manni *et al.*, 1995; Suh *et al.*, 1997). High polyamine levels are also associated with neuro-degenerateive disease including Alzheimer's (Morrison and Kish, 1995; Choi *et al.*, 2001). Polyamine biosynthesis in mammalian cells begins with putrescine derived from ornithine catalyzed by ornithine decarboxylase (ODC). The subsequent addition of an aminopropyl group to putrescine leads to the synthesis of spermidine, and the further addition of another aminopropyl group synthesizes spermine (Thomas and Thomas, 2001; Casero and Pegg, 1993).

Analysis of major polyamines (putrescine, spermidine, spermine) and their acetyl forms in various biological specimens obtained from cancer patients has shown altered polyamine biosynthesis and accumulation (Suh *et al.*, 1997; Kingsnorth and Wallace, 1985; Seiler *et al.*, 1987; Abdel-Monem and Ohno, 1978; Della *et al.*, 1983; Russell, 1977; Loser *et al.*, 1990a and b). In previous studies, increased polyamine levels have provided a diagnostic tool to evaluate malignant tumor activity. The accurate detection and identification of all the polyamines simultaneously in a single analysis is therefore becoming more important for the study of their biochemical roles.

Many analytical methods have been developed based on gas chromatography (GC) and liquid chromatography (LC) combined with selective detectors or mass spectrometry (MS) for polyamine analysis (Hiramatsu *et al.*, 1995; Loser *et al.*, 1988; Leveque *et al.*, 2000; Fu *et al.*, 1998; Rattenbury *et al.*, 1979; van den Berg *et al.*, 1986). In GC analysis, it is a prerequisite to block active hydrogen atoms in amino groups (Suh *et al.*, 1997; Lee *et al.*, 1998; Smith and Daves, 1977). However, these procedures are less suitable for polyamines than for the

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Abbreviations used: ODC, ornithine decarboxylase.

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other amines. In previous polyamine analysis, extractive carbamoylation with isobutyl chloroformate was found to be efficient in the recovery of polyamines (Choi *et al.*, 2000). The present study was undertaken to perform extractive carbamoylation of polyamines for the investigation of their biological roles in both urine and serum samples obtained from breast cancer patients. This was done by LC-MS analysis and fully validated as an alternative analytical technique in clinical applications.

EXPERIMENTAL

Chemicals. 1,3-Diaminopropane [1,3-Dap], putrescine [Put], cadaverine [Cad], *N*-acetylputrescine [*N*-actPut], *N*-acetylcadaverine [*N*-actCad], spermidine [Spd], *N*-acetylspermidine [*N*-actSpd], spermine [Sp], N^1-acetylspermine [N^1-actSp] and 1,6-diaminohexane as an internal standard (IS) were purchased from Sigma-Aldrich (St Louis, MO, USA). The HPLC-grade diethyl ether, *n*-pentane and methanol were obtained from J.T. Backer (Phillipsburg, NJ, USA). Isobutyl chloroformate was also purchased from Sigma-Aldrich. Each stock solution of polyamines was prepared at a 1000 µg/mL concentration in methanol and stored at −20°C until used. These stocks were used to prepare working solutions of various concentrations from 0.01 to 10 µg/mL, with methanol.

Sample collection. Urine and serum samples were collected from the Samsung Hospital (Seoul, Korea) and College of Medicine at Hanyang University (Seoul, Korea). Urine samples including breast cancer patients (ages 50.1 ± 8.0, *n* = 30) and normal subjects (ages 50.1 ± 7.9, *n* = 30) and serum samples including breast cancer patients (ages 47.1 ± 5.8, *n* = 30) and normal female subjects (ages 48.9 ± 8.3, *n* = 30) were tested. The Ethics Committee of the Samsung Hospital (Seoul, Korea) and College of Medicine at Hanyang University (Seoul, Korea) approved the study. All patients provided written informed consent before entry into the study. All urine and serum samples were stored at −20°C until analysis. Urinary creatinine values were determined using the Jaffé method.

Preparation of urine sample. A 200 µL urine sample was extracted for 10 min with 1 mL of pentane, and the organic phase was discarded by centrifugation (2500 rpm, for 5 min). After 20 µL 1,6-diaminohexane (1 µg/mL) was added, the sample was adjusted to a pH 9.0 by adding 70 µL sodium-carbonate buffer (0.1 M, pH 9.0). Amine carbamoylation was performed by adding 20 µL isobutyl chloroformate followed by standing for 15 min at 35°C. After cooling, the solution was extracted with 2 mL of diethyl ether twice, and the organic solvent combined was evaporated under a gentle nitrogen steam at room temperature. The residue was reconstituted with 100 µL of the mixture of 0.2% acetic acid and 0.2% acetic acid–acetonitrile (50:50, v/v), and a 30 µL aliquot was injected into the LC–MS system.

Preparation of serum sample. A 200 µL serum sample was diluted with 1 mL of deionized water and mixed for 1 min. After heating at 60°C for 20 min to precipitate the protein, 50 µL IS (0.1 µg/mL) was added and the sample was adjusted

to a pH 9.0 by adding 25 µL sodium-carbonate buffer (1.0 M, pH 9.0). The aqueous sample was extracted and derivatized in the same manner as described for urine sample.

Liquid chromatography–mass spectrometry. Chromatography was performed with a Shiseido nanospace SI-2 HPLC system (Shiseido Co., Tokyo, Japan) coupled to a Shiseido MG C18 column (5 µm, 150 × 1.5 mm i.d.). A gradient eluent (A, 0.2% acetic acid; B, 0.2% acetic acid in acetonitrile) at 100 µL/min was used.

All data were recorded on a Thermo LCQ advantage iontrap MS equipped with electrospray ionization (ESI; Thermo, San Jose, CA, USA) operated in the positive ionization mode. The operating conditions were set as follows: spray voltage, 6 kV; capillary voltage, 4 V; tube lens offset voltage, 40 V; sheath gas flow rate, 30 units; and capillary temperature, 250°C. In tandem MS analysis, the protonated molecular ions were fragmented by helium gas collisions.

Method validation. The urinary concentration ranges for the calibration curve were 1 to 5000 ng/mL for *N*-actPut and *N*-actSpd, 1–2500 ng/mL for *N*-actCad and 1–250 ng/mL for the other six polyamines studied. In serum analysis the range was set at 1–250 ng/mL for all polyamines. The calibration curves were calculated by the peak area ratio (compound area/IS area) and the limit of detection (LOD) was determined at a signal-to-noise ratio of 3. The low, medium and high concentrations were assessed to validate the method in both urine and serum, and repeated on five different days with three batches.

Evaluation of matrix effects. The matrix effect using the standard addition method was investigated based on a previous report (Cho *et al.*, 2006). Blank and spiked sample with (blank + X) along with calibration standards were analyzed. Using the calibration standards, one can obtain the observed concentration of blank and blank + X. The expected blank + X can be obtained by adding the observed blank and amount of X added. The matrix effects can be evaluated by comparing the observed blank + X with the expected blank + X. Urine and serum samples obtained from normal subjects were used as blanks. In urine, the matrix effect was performed at 10 and 100 ng/mL for 1,3-Dap, Put, Cad, N^1-actSp, Spd and Sp, and at 100 and 1000 ng/mL for *N*-actPut, *N*-actSpd and *N*-actCad. In the serum, it was also tested at 10 and 50 ng/mL for all compounds.

RESULTS AND DISCUSSION

Derivatization of polyamines

Carbamoylation with isobutyl chloroformate was introduced to analyze polyamines in both urine and serum samples. When sample preparation is employed for the polyamine analysis in urine and serum samples, complex purification steps are required to reduce interference coming from the sample matrices (Suh *et al.*, 1997; Lee *et al.*, 1998; Smith and Daves, 1977). In our recent experiment (Choi *et al.*, 2000), carbamoylation
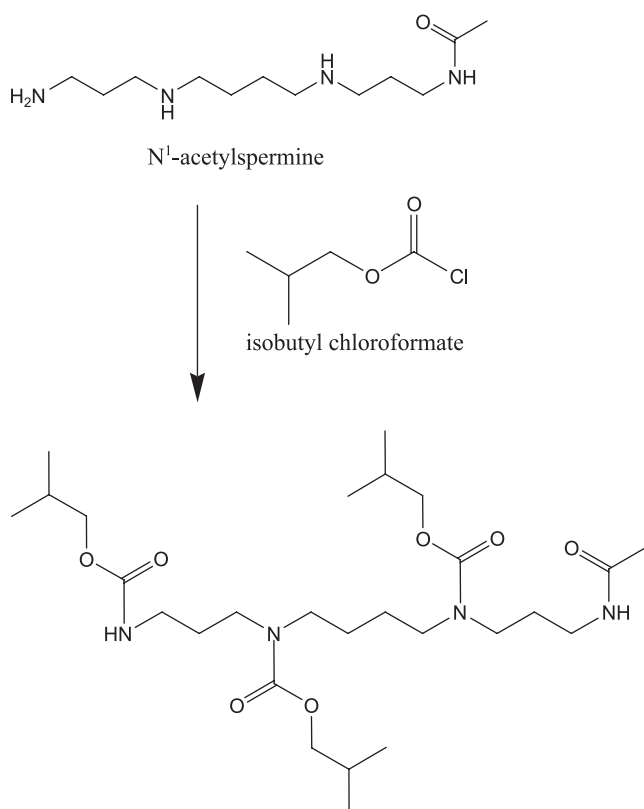
Figure 1. The carbamoylation with isobutyl chloroformate of N^1-acetylspermidine.
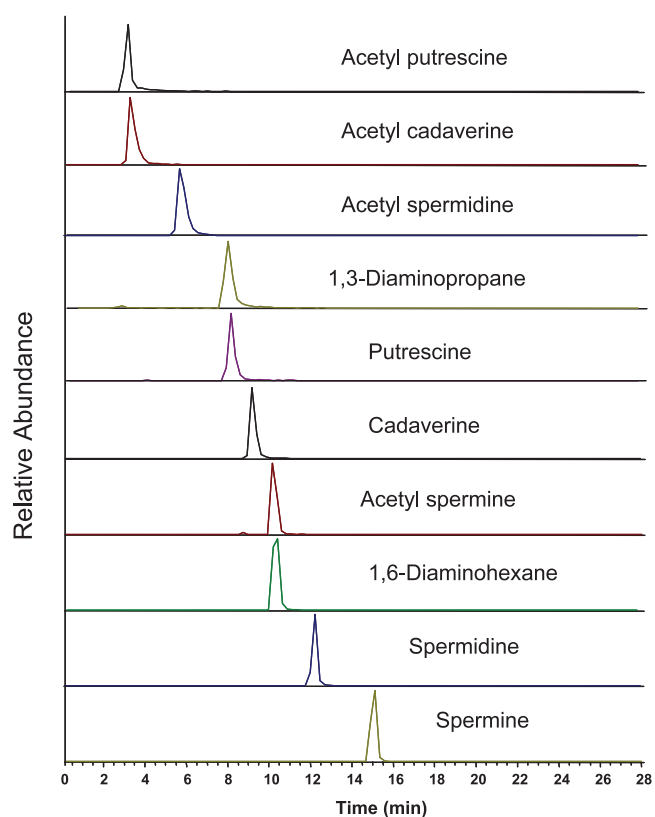

Figure 2. The LC-MS/MS chromatograms for 9 polyamines and 1,6-diaminohexane as an internal standard in the positive ionization mode. This figure is available in colour online at www.interscience.wiley.com/journal/bmc

was effective for polyamine analysis, but it required additional derivatization in GC-MS analysis. Accordingly, it was desirable to improve the detectability without complex sample preparation steps and the carbamoylation was accomplished with LC-MS analysis in this study (Fig. 1). Under the present extraction using diethyl ether, all polyamines were derivatized and analyzed without significant interference from both urine and serum samples (Fig. 2).

LC-ESI-MS/MS analysis

All polyamines were protonated in the mass spectra as their carbamoyl derivatives. The $[M + H]^+$ ions were used as precursor ions in the MS/MS analysis (Table 1). For the generation of MS/MS spectra, $[M + H]^+$ precursor ions were fragmented at an optimal collision energy of 25% (Fig. 3).

Method validation

In urine, the calibration curves showed correlation coefficients (r^2) > 0.995 in the range 1–250 ng/mL for 1,3-Dap, Put, Cad, Spd, Sp and N^1-actSp, 1–2500 ng/mL for N-actCad and 1–5000 ng/mL for N-actPut, N-actSpd (Table 2). The coefficient of variation (CV, %) for intra-day and inter-day variations ranged from 1.0 to

Table 1. Collision-induced dissociation of the protonated polyamine derivatives in the positive ionization mode

Polyamines	Precursor ion (m/z)	MS/MS product ion (m/z)
N-actPut	231.0	157.0
N-actCad	245.0	171.1
N-actSpd	388.0	314.1
1,3-Dap	275.0	201.0
Put	289.0	215.0
Cad	303.0	229.0
N^1-actSp	545.3	471.3
Spd	446.1	372.1
Sp	603.0	529.2
IS	317.0	243.0

Table 2. Calibration curves for urinary polyamines analysis

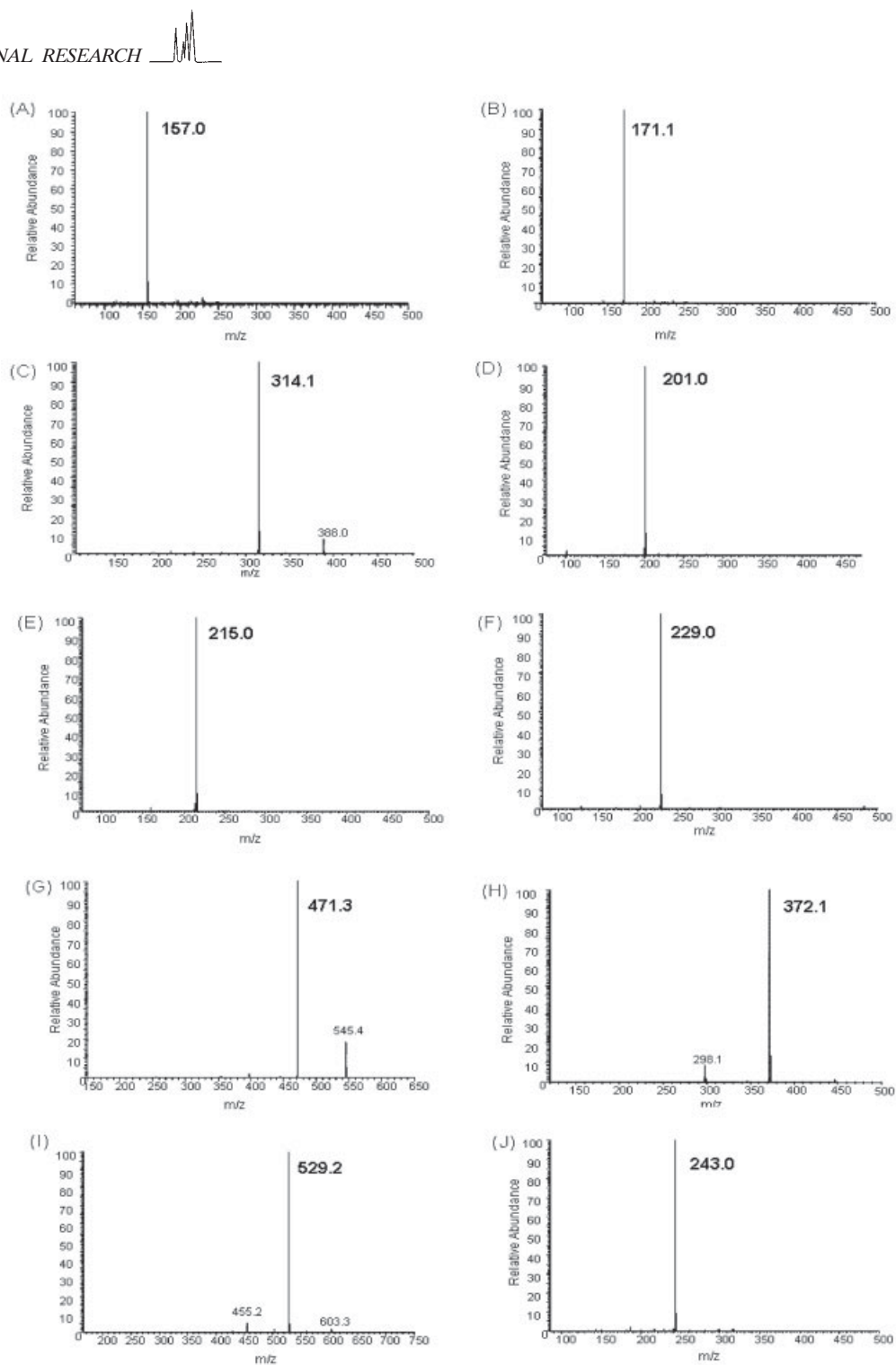| Polyamines	Calibration curve $Y = aX + b$		Linearity (r^2)
	a	b	
N-actPut	0.0007	0.0082	0.9949
N-actCad	0.0009	0.0135	0.9994
N-actSpd	0.0094	0.0189	0.9997
1,3-Dap	0.0058	0.0271	0.9952
Put	0.0034	0.0059	0.9994
Cad	0.0053	−0.0134	0.9959
N^1-actSp	0.0044	−0.0034	0.9958
Spd	0.0152	−0.0163	0.9991
Sp	0.006	0.0045	0.9996

Figure 3. The MS/MS spectra of (A) *N*-acetylputrescine, (B) *N*-acetylcadaverine, (C) *N*-acetylspermidine, (D) 1,3-diaminopropane, (E) putrescine, (F) cadaverine, (G) N^1-acetylspermine, (H) spermidine, (I) spermine and (J) 1,6-diaminohexane as an internal standard.

Table 3. Intra- and inter-day assays of urinary polyamine analysis

Polyamines	Concentration added (ng/mL)	Intra-day ($n = 3$) Amount found (mean ± SD[a])	CV[b] (%)	Inter-day ($n = 5$) Amount found (mean ± SD[a])	CV[b] (%)
1,3-Dap	5.0	5.5 ± 0.7	13.1	5.9 ± 0.9	14.6
	50.0	46.9 ± 2.8	6.0	48.5 ± 3.6	7.3
	100.0	99.7 ± 3.4	3.4	100.1 ± 1.8	1.8
Put	5.0	7.0 ± 0.7	10.3	6.7 ± 0.5	7.2
	50.0	44.7 ± 4.5	3.7	46.0 ± 4.0	8.6
	100.0	97.3 ± 6.0	6.2	98.2 ± 6.1	6.2
Cad	5.0	4.3 ± 1.8	14.8	4.3 ± 0.6	14.5
	50.0	45.5 ± 1.5	3.2	47.1 ± 3.6	7.7
	100.0	101.8 ± 13.8	13.6	100.9 ± 10.1	10.0
N^1-actSp	5.0	5.0 ± 1.3	10.0	5.0 ± 0.5	9.9
	50.0	51.5 ± 3.8	7.4	49.0 ± 2.1	4.4
	100.0	101.3 ± 1.0	1.0	101.0 ± 1.4	1.4
Spd	5.0	4.8 ± 0.6	12.2	3.3 ± 0.5	15.0
	50.0	50.5 ± 4.8	9.5	46.2 ± 6.2	13.4
	100.0	92.9 ± 8.5	8.6	95.4 ± 8.4	8.8
Sp	5.0	5.7 ± 0.8	14.9	5.6 ± 0.8	13.4
	50.0	45.9 ± 4.7	10.3	47.7 ± 4.2	8.9
	100.0	102.1 ± 2.4	2.3	101.9 ± 1.7	1.7
N-actPut	50.0	44.9 ± 1.9	4.1	48.8 ± 7.0	14.3
	250.0	230.6 ± 17.2	7.5	246.4 ± 27.7	11.2
	1000.0	1015.9 ± 97.4	9.6	1096.6 ± 42.4	3.9
	2500.0	2470.4 ± 80.7	3.3	2469.9 ± 79.9	3.2
N-actCad	50.0	43.6 ± 3.3	7.7	45.8 ± 5.1	11.1
	250.0	214.0 ± 25.8	12.1	231.5 ± 30.0	13.0
	1000.0	1026.2 ± 87.0	8.5	1037.2 ± 74.2	7.2
	2500.0	2603.8 ± 179.2	6.9	2504.6 ± 29.0	1.2
N-actSpd	50.0	53.0 ± 0.8	1.5	51.9 ± 7.4	14.3
	250.0	240.0 ± 25.4	10.6	247.2 ± 29.0	11.7
	1000.0	1061.2 ± 112.8	10.6	1091.7 ± 60.7	5.6
	2500.0	2557.3 ± 131.9	5.2	2527.4 ± 153.6	6.1

[a] SD = standard deviation. [b] CV = coefficient of variation.

14.9%, and from 1.2 to 15.0%, respectively (Table 3). In the serum, the calibration curves showed correlation coefficients (r^2) > 0.994 in the range 1–250 ng/mL for all polyamines (Table 4). The coefficient of variation (CV, %) for intra-day and inter-day variations ranged from 3.1 to 13.7%, and from 3.1 to 15.0%, respectively (Table 5). The limit-of-quantification (LOQ) was 1 ng/mL based on a signal-to-noise ratio of >3 in both urine and serum analysis.

Matrix effects

The matrix effect ranged from 0.04 to 13.8% in urine and from 2.4 to 14.7% in serum. The matrix effect was less than 15% in both urine and serum samples, and the present method was therefore conducted for quantitative analysis of polyamines.

Quantification of polyamines in clinical applications

The polyamine concentrations in urine and serum obtained from 30 patients with breast cancer were

Table 4. Calibration curves for polyamines in serum

Polyamines	Calibration curve $Y = aX + b$ a	b	Linearity (r^2)
N-actPut	0.0021	−0.0340	0.9968
N-actCad	0.0034	−0.0059	0.9983
N-actSpd	0.0409	−0.0197	0.9981
1,3-Dap	0.0245	−0.034	0.9963
Put	0.0518	0.1184	0.9978
Cad	0.0238	−0.0365	0.9966
N^1-actSp	0.0181	0.0289	0.9939
Spd	0.0650	−0.1586	0.9960
Sp	0.0210	0.0058	0.9972

measured, and compared with 30 normal controls. In previous results, the polyamine levels in tissue, urine and serum of patients were significantly higher than in normal tissue, urine and serum. In contrast, the polyamine levels of breast cancer patients were higher only in tissue, and there was no significant difference in the urine between breast cancer patients and the controls in other reports (Leveque *et al.*, 2000; Romano *et al.*, 1981). In this study, the results from urinary

Table 5. Intra- and inter-day assays of polyamine analysis in serum

Polyamines	Concentration added (ng/mL)	Intra-day (n = 3)		Inter-day (n = 5)	
		Amount found (mean ± SD[a])	CV[b] (%)	Amount found (mean ± SD[a])	CV[b] (%)
1,3-Dap	5.0	5.3 ± 0.7	13.4	4.9 ± 0.7	15.0
	50.0	53.3 ± 4.8	9.0	56.1 ± 4.7	8.4
	100.0	103.7 ± 9.5	9.1	106.0 ± 7.3	6.8
Put	5.0	6.3 ± 0.9	14.2	5.2 ± 0.7	13.8
	50.0	48.7 ± 5.0	10.3	51.3 ± 2.7	5.2
	100.0	101.3 ± 8.4	8.2	104.6 ± 10.0	9.6
Cad	5.0	4.6 ± 0.6	13.5	5.2 ± 0.7	13.2
	50.0	51.9 ± 4.9	9.5	53.3 ± 6.8	12.7
	100.0	99.3 ± 12.2	12.2	97.4 ± 10.5	8.6
N^1-actSp	5.0	5.0 ± 0.6	12.7	5.6 ± 0.7	13.1
	50.0	48.3 ± 5.5	11.4	48.3 ± 4.2	8.7
	100.0	94.6 ± 12.9	13.6	93.6 ± 4.0	4.2
Spd	5.0	5.6 ± 0.6	10.4	4.7 ± 0.2	3.4
	50.0	48.6 ± 5.1	10.5	47.6 ± 3.6	7.5
	100.0	91.4 ± 6.7	7.3	99.1 ± 8.8	12.0
Sp	5.0	5.0 ± 0.7	13.3	5.0 ± 0.6	12.6
	50.0	47.0 ± 3.8	7.8	47.1 ± 5.2	11.0
	100.0	109.2 ± 3.3	3.1	105.6 ± 6.5	6.2
N-actPut	5.0	4.9 ± 0.7	13.7	4.9 ± 0.2	3.1
	50.0	52.8 ± 5.0	9.4	53.6 ± 7.5	13.9
	100.0	98.9 ± 9.3	9.4	99.0 ± 8.5	8.6
N-actCad	5.0	5.3 ± 0.7	12.7	4.4 ± 0.3	7.5
	50.0	50.5 ± 3.1	6.2	54.5 ± 4.7	8.7
	100.0	95.1 ± 7.0	7.4	101.8 ± 8.8	8.6
N-actSpd	5.0	6.1 ± 0.6	9.3	6.0 ± 0.8	12.7
	50.0	51.0 ± 7.0	13.7	51.5 ± 5.8	11.3
	100.0	100.1 ± 8.3	8.3	99.5 ± 9.5	9.5

[a] SD = standard deviation. [b] CV = coefficient of variation.

Table 6. Concentrations of urinary polyamines measured (n = 30)

Polyamines	Concentration (mean ± SD[a]) (μmol/g of creatinine)		
	Normal (n = 30)	Patients (n = 30)	p-Value
N-actPut	16.9 ± 12.9	9.4 ± 9.4	<0.05
N-actCad	7.1 ± 17.4	0.9 ± 1.1	NS
N-actSpd	9.22 ± 2.69	1.8 ± 2.1	<0.05
1,3-Dap	0.02 ± 0.01	0.04 ± 0.04	NS
Put	0.4 ± 0.6	0.4 ± 0.5	NS
Cad	0.5 ± 1.3	0.2 ± 0.3	NS
N^1-actSp	0.04 ± 0.05	0.01 ± 0.03	<0.01
Spd	0.2 ± 0.3	0.08 ± 0.06	<0.02
Sp	0.08 ± 0.1	0.04 ± 0.09	NS

[a] SD = standard deviation.

polyamine analysis were in accordance with previous reports (Leveque *et al.*, 2000; Romano *et al.*, 1981). Breast cancer is a local disease and exhibits low polyamine concentrations in comparison with prostatic (Cipolla *et al.*, 1990) and colonic tissues (Loser *et al.*, 1990a,b). Therefore, in the case of breast cancer, the urinary polyamine level would not be a good tumor marker in diagnosis or prognosis, unlike in other cancers (Table 6). In serum, 1,3-Dap, Put, Sp, and *N*-

actSpd levels significantly increased in breast cancer patients (Table 7). These results are well matched with previous reports that the serum concentrations of Put and Sp in breast cancer patients are higher than in controls (Nishioka and Romsdahl, 1974; Inamdar *et al.*, 1988). In our study, the serum level of *N*-actSpd was also higher in breast cancer patients. From these results, 1,3-Dap, Put, Sp, and *N*-actSpd in serum could be potent diagnostic biomarkers of breast cancer.

Table 7. Concentration of polyamines measured in serum

Polyamines	Concentration (mean ± SD) (ng/mL)		
	Normal ($n = 30$)	Patient ($n = 30$)	p-Value
N-actPut	13.49 ± 12.53	9.07 ± 12.94	NS
N-actCad	1.77 ± 1.68	2.10 ± 1.42	NS
N-actSpd	9.22 ± 2.69	11.92 ± 5.73	<0.05
1,3-Dap	3.00 ± 2.11	9.94 ± 9.53	<0.005
Put	26.04 ± 19.75	37.95 ± 18.04	<0.05
Cad	2.17 ± 0.58	1.61 ± 1.10	NS
N^1-actSp	1.29 ± 0.38	1.06 ± 0.52	NS
Spd	15.97 ± 14.15	20.31 ± 16.79	NS
Sp	1.90 ± 1.61	4.29 ± 2.87	<0.005

[a] SD = standard deviation.

CONCLUSION

In the present study, we developed a quantitative method for urinary and serum polyamine combining carbamoylation and LC-MS/MS analysis. This method is very simple and rapid with reliable accuracy and precision, while the limits of quantification were down to 1 ng/mL in both urine and serum analysis. The method was used for monitoring the polyamine concentration range in urine and serum samples obtained in 30 breast cancer patients and 30 age- and gender-matched normal controls. There was no significant difference between the urine of breast cancer patients and the controls in urinary polyamine levels. In contrast, the concentrations of 1,3-Dap, Put, Sp, and N-actSpd levels in serum were significantly increased in breast cancer patients.

Acknowledgements

REFERENCES

Abdel-Monem MM and Ohno K. Polyamine metabolism III: urinary acetyl polyamines in human cancer. *Journal of Pharmaceutical Sciences* 1978; **67**: 1671–1673.

Casero RA Jr and Pegg AE. Spermidine/spermine N1-acetyltransferase—the turning point in polyamine metabolism. *The FASEB Journal* 1993; 7: 653–661.

Chattopadhyay MK, Tabor CW and Tabor H. Polyamines protect Escherichia coli cells from the toxic effect of oxygen. *Proceedings of the National Academy of Sciences of the United States of America* 2003; **100**: 2261–2265.

Cho SH, Lee J, Lee WY and Chung BC. Direct determination of acylcarnitines in amniotic fluid by column-switching liquid chromatography with electrospray tandem mass spectrometry. *Rapid communications in mass spectrometry* 2006; **20**: 1741–1746.

Choi MH, Kim KR and Chung BC. Determination of hair polyamines as *N*-ethoxycarbonyl-*N*-pentafluoropropionyl derivatives by gas chromatography–mass spectrometry. *Journal of Chromatography A* 2000; **897**: 295–305.

Choi MH, Kim KR, Kim IS, Lho DS and Chung BC. Increased hair polyamine levels in patients with Alzheimer's disease. *Annals of Neurology* 2001; **50**: 128.

Cipolla B, Moulinoux JP, Quemener V, Havouis R, Martin LA, Guille F and Lobel B. Erythrocyte polyamine levels in human prostatic carcinoma. *Journal of Urology* 1990; **144**: 1164–1166.

Della Ragione F and Pegg AE. Studies of the specificity and kinetics of rat liver spermidine/spermine N1-acetyltransferase. *Biochemical Journal* 1983; **213**: 701–706.

Fu S, Zou X, Wang X and Liu XJ. Determination of polyamines in human prostate by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography B: Biomedical Sciences and Applications* 1998; **709**: 297–300.

Hiramatsu K, Sugimoto M, Kamei S, Hoshino M, Kinoshita K, Iwasaki K and Kawakita M. Determination of amounts of polyamines excreted in urine: demonstration of N1,N8-diacetylspermidine and N1,N12-diacetylspermine as components commonly occurring in normal human urine. *Journal of biochemistry (Tokyo)* 1995; **117**: 107–112.

Inamdar NA, Redkar SL, Mittra I and Damle SR. Clinical significance of serum spermine in breast cancer. *Tumori* 1988; **74**: 171–176.

Kingsnorth AN and Wallace HM. Elevation of monoacetylated polyamines in human breast cancers. *European Journal of Cancer and Clinical Oncology* 1985; **21**: 1057–1062.

Lee SH, Kim SO, Lee HD and Chung BC. Estrogens and polyamines in breast cancer: their profiles and values in disease staging. *Cancer Letters* 1998; **133**: 47–56.

Leveque J, Foucher F, Bansard JY, Havouis R, Grall JY and Moulinoux JP. Polyamine profiles in tumor, normal tissue of the homologous breast, blood, and urine of breast cancer sufferers. *Breast Cancer Research and Treatment* 2000; **60**: 99–105.

Loser C, Wunderlich U and Folsch UR. Reversed-phase liquid chromatographic separation and simultaneous fluorimetric detection of polyamines and their monoacetyl derivatives in human and animal urine, serum and tissue samples: an improved, rapid and sensitive method for routine application. *Journal of Chromatography B: Biomedical Sciences and Applications* 1988; **430**: 249–262.

Loser C, Folsch UR, Paprotny C and Creutzfeldt W. Polyamine concentrations in pancreatic tissue, serum, and urine of patients with pancreatic cancer. *Pancreas* 1990a; **5**: 119–127.

Loser C, Folsch UR, Paprotny C and Creutzfeldt W. Polyamines in colorectal cancer. Evaluation of polyamine concentrations in the colon tissue, serum, and urine of 50 patients with colorectal cancer. *Cancer* 1990b; **65**: 958–966.

Manni A, Grove R, Kunselman S and Aldaz M. Involvement of the polyamine pathway in breast cancer progression. *Cancer Letters* 1995; **92**: 49–57.

Morrison LD and Kish SJ. Brain polyamine levels are altered in Alzheimer's disease. *Neruroscience Letters* 1995; **197**: 5–8.

Nishioka K and Romsdahl MM. Elevation of putresine and spermidine in sera of patients with solid tumors. *Clinica Chimica Acta* 1974; **57**: 155–161.

Parham DM and Robertson AJ. A retrospective study of breast carcinoma: causes of death and pattern of metastases. *British Journal of Cancer* 1989; **60**: 394–396.

Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Research* 1988; **48**: 759–774.

Rattenbury JM, Lax PM, Blau K and Sandler M. Separation and quantification of urinary di- and polyamines by gas chromatography with electron capture detection. *Clinica Chimica Acta* 1979; **95**: 61–67.

Romano M, Cecco L and Cerra M. Levels of polyamines and nucleic acids in human breast carcinoma. *Tumori* 1981 **67**: 431–435.

Russell DH. Clinical relevance of polyamines as biochemical markers of tumor kinetics. *Clinical Chemistry* 1977; **23**: 22–27.

Seiler N. Functions of polyamine acetylation. *Canadian Journal of Physiology and Pharmacology* 1987; **65**: 2024–2035.

Seiler N, Bolkenius FN and Rennert OM. Interconversion, catabolism and elimination of the polyamines. *Medical Biology* 1981; **56**: 334–346.

Smith RG and Daves GD. Gas chromatography mass spectrometry analysis of polyamines using deuterated analogs as internal standards. *Biomedical Mass Spectrometry* 1977; **4**: 146–151.

Suh JW, Lee SH, Chung BC and Park J. Urinary polyamine evaluation for effective diagnosis of various cancers. *Journal of chromatography. B, Biomedical Sciences and Applications* 1997; **688**: 179–186.

Thomas T and Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cellular and Molecular Life Sciences* 2001; **58**: 244–258.

van den Berg GA, Muskiet FA, Kingma AW, van der Slik W and Halie MR. Simultaneous gas-chromatographic determination of free and acetyl-conjugated polyamines in urine. *Clinical Chemistry* 1986; **32**: 1930–1937.