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Review

Polyamines as cancer markers: applicable separation methods

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Abstract

Spermine, spermidine, putrescine and cadaverine are aliphatic amines widely spread in the human body. Their concentrations together with their acetyl conjugates increase significantly in the biological fluids and the affected tissues of cancer patients. Their concentrations decrease with the improvement in the patient's condition on multiple therapy. Various chromatographic techniques are frequently used in monitoring concentrations of di- and polyamines in cancer. Among these techniques, thin-layer chromatography and liquid chromatography using pre- or postcolumn derivatization, separating on a reversed-phase or an ion-exchange column are the most commonly used. Besides, high-resolution capillary column gas chromatography (GC) is increasingly used over packed column GC, and in recent years, capillary zone electrophoresis has also gained some importance in polyamine determinations. The review examines the prospects and the limitations of polyamines as cancer markers using chromatographic and electrophoretic techniques.

Keywords: Reviews; Polyamines; Spermine; Spermidine; Putrescine; Cadaverine

Contents

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1. Introduction

The polyamines sperimine (Spm) and spermidine (Spd) and diamines putrescine (Put) and cadaverine (Cad) are constituents of eukaryotic and prokaryotic cells, having multiple functions in living organisms [1]. Polyamines and their metabolites are considered to be involved in the process of cell multiplication and its regulation [2,3]. Rapidly growing tissues usually have higher amounts of polyamines and they have a stimulating effect on DNA, RNA and protein synthesis. Conversely, severe depletion of polyamines reduces growth in mammalian cells [4,5]. Polyamines are reported to have a role in the prevention of nerve damage [6,7] and duodenal mucosal repair [8]. Spm has been recommended for the treatment of human prostrate cancer separately and in combination with other anticancer drugs [9,10]. Wallance [11] has indicated the functions of the polyamines as growth factors; antioxidants; stabilizers of DNA, RNA, membranes; metabolic regulators; nutrients and second messengers.

Table 1
Determination of polyamines in normal human urine

Age (yr)	Sex (M/F)	Creatinine (mg/ml)	Polyamine μg/mg creatinine		
			Put	Spd	Spm
33	M	1.465	1.787	0.303	0.881
26	M	1.419	0.912	0.307	0.426
53	M	1.600	0.540	0.266	0.499
29	F	1.364	0.431	0.288	0.438
20	M	1.958	0.479	0.747	0.777
40	F	1.905	0.940	+	0.299
13	F	1.638	2.116	0.055	0.322
25	M	1.389	2.422	0.175	+
48	M	1.097	2.112	0.025	0.559
Average			1.304	0.241	0.467
SD			0.799	0.227	0.262

+ Under the level of determination.
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Put, Cad, Spd and Spm are polycations under physiological conditions and exhibit net charges close to 2+, 2+, 3+ and 4+, respectively. As polycations, the polyamines could interact electrostatically with negatively charged moieties such as DNA, RNA, proteins and phospholipids and stabilize their structure. Spd and Spm are particularly flexible and have charge distribution along the whole molecule, thus facilitating greater interaction with the negatively charged backbone of DNA [12,13].

The significant increase in the level of polyamines of cancer patients is reported which is considered due to either increased secretion from the proliferating cells themselves or release from dead cells, as a consequence of the active replacement of cell growing tissues [14–16]. However, their suitability as cancer markers is challenged because polyamines are also elevated in association with benign disorders [17].

Free and acetylated Spd, Spd and their precursor Put have been reported in mammalian tissue, serum, urine [18] (Table 1) and the central nervous system [6]. The acetyl derivatives are commonly observed in human urine samples [19,20] (Table 2). Cad is produced by the decarboxylation of lysine and has been considered to have a role in prokaryotic cells, but Benamouziq et al. [21] have observed high concentration of Cad in human jegunum and Loser et al. [17] in colorectal cancer patients. Free and acetylated polyamines are shown in Figs. 1 and 2.

2. Biosynthesis

All eukaryotic cells synthesize Put, Spd and Spm and contain a low level of Put and higher levels of Spd and Spm [22]. The principal precursors of the polyamines are amino acids L-ornithine and L-methonine [1–3]. The initial hydrolysis of L-arginine to L-ornithine and urea, catalysed by arginase (L-arginine amidino hydrolase) may also be considered.

Table 2
Polyamines in urine of healthy persons

Age (yr)	Mean (mol/g creatinine)	SD	RSD (%)
Total	22.2	6.1	27
Put	0.54	0.52	97
AcPut	9.57	3.52	37
Cad	0.23	0.24	105
Spd	0.17	0.14	80
AcCad	5.55	2.64	48
N^1-AcSpd	2.70	0.84	31
N^8-AcSpd	2.14	0.61	25
Dacspd	0.301	0.109	36
Spm	0.62	0.98	158
AcSpm	0.032	0.077	237
Dacspm	0.102	0.017	48

Urine samples from 52 apparently healthy volunteers were analyzed. SD=Standard deviation, RSD=relative standard deviation.
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Put is formed by direct decarboxylation of L-ornithine. The reaction is catalysed by ornithine decarboxylase (ODC) and may be a rate determining step in polyamine synthesis in nonproliferating cells. However, in rapidly dividing cells (e.g., embryonal cells, cells of gut mucosa, tumor cells) ODC activity is higher and is not considered a rate limiting parameter [19,20]. ODC requires pridoxal 5′-phosphate for its catalytic activity [22], and is characterized by a short half life (20 min) [1,6,23].

Spd and Spm are synthesized from Put by the action of spermidine synthase and sperimine synthase, where the reaction involves the addition of aminopropyl to Put or Spd, respectively. The aminopropyl groups are ultimately derived from L-methionine. L-Methionine is converted to *S*-adenosyl-L-methionine (Adomet) by L-methionine-*S*-

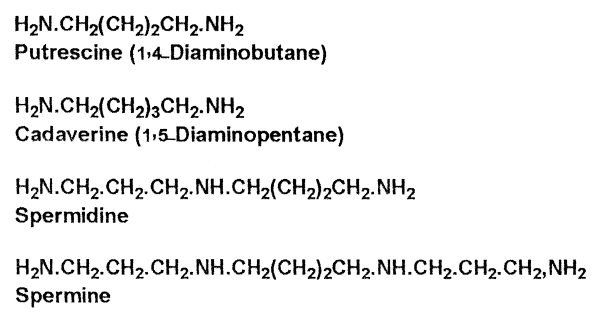

Fig. 1. Structures of naturally occurring polyamines.

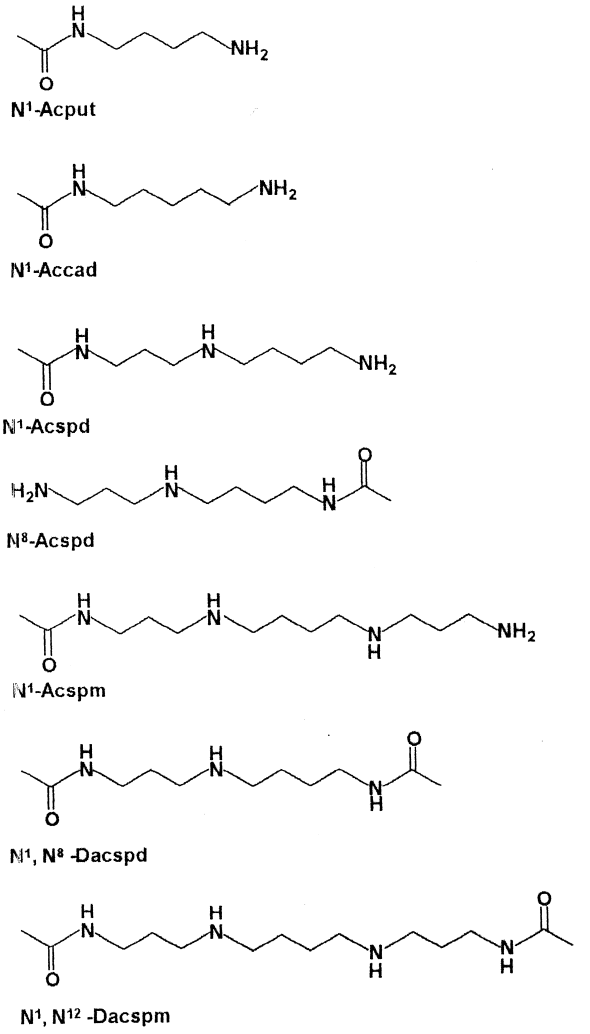

Fig. 2. Structures of naturally occurring mono- and diacetylpolyamines.

adenosyltransferase. Decarboxylation of Adomet to aminopropyl donor is catalysed by *S*-adenosylmethionine decarboxylase (Adometdc). Adomet after decarboxylation is committed to polyamine biosynthesis [4,23,24] (Fig. 3). The concentration of decarboxylated Adomet is normally very low in mammalian cells and the substrate Adometdc which produces it is activated by Put and is therefore, a key step in polyamine production [3,25].

Polyamine is catabolized and degraded back to Put by the action of polyamine oxidase (PAO) and spermidine/spermine N^1-acetyltransferase [26] (Fig.

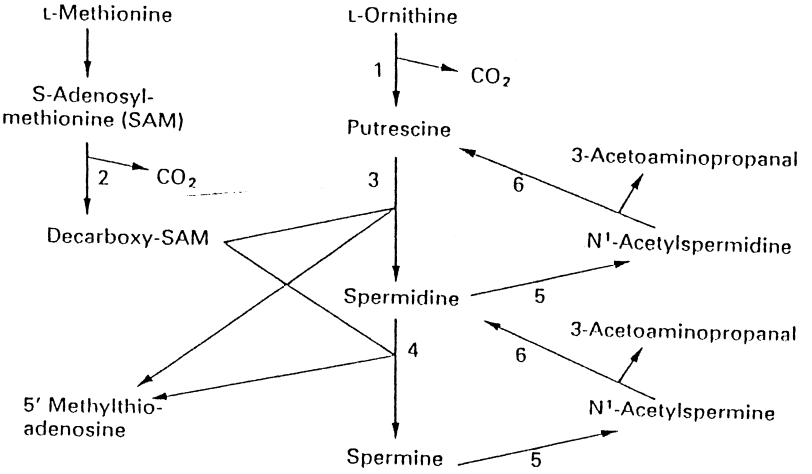

Fig. 3. Polyamine metabolic pathways in mammalian cells (1) ornithine decarboxylase, (2) *S*-adenosylmethionine decarboxylase, (3) spermidine synthase, (4) spermine synthase, (5) spermidine/spermine N^1-acetyltransferase, (6) polyamine oxidase. From Ref. [23], with permission.

3). The latter converts Spm and Spd to N^1-acetylspermine (N^1-Acspm) and N^1-acetylspermidine (N^1-Acspd) which are converted to Spd and Put, respectively, by PAO after splitting off 3-acetamidopropanal [27]. The activity of acetyltransferase is normally low and may be a rate limiting factor in the interconversion [3]. However, its activity could be induced by introducing exogenous polyamines [4]. The acetylase/oxidase system may be a regulatory response that act to reduce intracellular polyamine contents, once it becomes too high [3]. Put formed is degraded by the action of diamine oxidase (DAO) or excreted from the cell [4].

3. Polyamines in cancer

Rapid tumor growth has been associated with remarkable altered polyamine biosynthesis and accumulation [28]. In solid tumors the activity of ODC and Adometdc is higher compared to normal tissue [28–31]. Consequently, the concentrations of Put and Spd are also enhanced considerably [32] (Table 3). ODC and Adometdc are generally regulated in the cell depending on stimuli affecting growth and cellular contents of Put and polyamines [4,33]. The activity of ODC is known to respond on the increased amount of protein in ODC [1]. It has been postulated that ODC and Adometdc have sequences corresponding to the PEST (proline–glutamatic and serine–threonine) region which is important in protein turnover [34]. ODC activity also depends on the properties of oncogens [35].

A number of studies have indicated higher concentration of Put, Spd and Spm or total polyamine contents (free and acetylated) in cancer patients compared to healthy subjects [13,14,36–40]. Lee et al. [41] observed higher mean levels of polyamines in serum of uterine cancer patients than those in normal serum [41]. Suh et al. [15] reported significant differences in urinary polyamines in advanced gastric carcinoma, ovarian cancer, acute myelocytic leukemia, non-Hodgkins lymphoma as compared to healthy subjects. The results of Saverio et al. [42] indicated that transcripts encoding ODC, ODC antizme Adometdc and Spd/Spm N^1-acetyltransferase were significantly higher whereas chusterin (sulfated glycoprotein′2) mRNA was significantly lower in tumor tissues compared with benign tissues. Bakowski et al. [43] observed that patients undergoing remission induction chemotherapy showed a significant elevated plasma Spd level within 48 h and suggested that tumor response to chemotherapy can be predicted on the basis of plasma polyamines. The

Table 3
Determination of polyamines in urine from cancer patients

Age (yr)	Sex (M/F)	Diagnosis	Polyamines (μg/mg creatinine)		
			Put	Spd	Spm
27	F	Breast cancer	2.910	1.521	1.056
49	M	Lung cancer	8.063	1.312	+
53	F	Melanoma	5.509	1.809	+
44	M	Ovary tumour	10.27	1.092	+
53	M	Lung cancer	12.49	4.632	10.45
73	M	Lung cancer	40.88	5.862	+
58	M	Lung cancer	8.80	2.62	+
63	F	Lung cancer	10.15	2.84	6.33
60	F	Lung cancer	288.0	8.80	+
56	M	Lung cancer	2.10	+	+
66	M	Lung cancer	1.70	1.46	+
29	M	Lung cancer	1.90	0.19	+
51	M	Lung cancer	29.0	13.0	8.71
64	M	Lung cancer	9.29	+	+
53	M	Lung cancer	21.60	43.8	+
50	M	Lung cancer	4.86	+	+
Average (n=16)			28.60	5.56	1.66
SD			70.0	10.7	3.3

+ Under the level of determination.
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increase in polyamines is explained on the basis of cell kill in a responsive tumors on chemotherapy. A similar response has also been reported with a sharp increase in urinary polyamines output on initial treatment by chemotherapy, surgery or radiotherapy [44–46]. In the responding patients, polyamine levels are shown to normalize when the patient is in remission. On the other hand, patients with a recurrent tumor or metastatic disease show higher polyamine excretion, thus the response of an individual can be monitored [23,28,47,48].

Nonresponders of the chemotherapy tended to show elevated Put without any significant increase in Spd. The plot of post to pretreatment Spd ratio over post to pre Put ratio had value of 1.4 for complete responders, 1.2 for partial responders and 0.4 for nonresponders [28].

Peng et al. [49] reported that total polyamines, especially the contents of Cad and Spd in malignant ascites were significantly higher as compared to the patients with cirrhosis and tuberculosis suggesting that ascite levels of polyamine can be regarded as one of the cancer markers.

Suh and co-workers [15,40] evaluated urinary polyamines for various cancers and observed that the ratio of both Put to Spd and total (free and acetylated) Put to total Spd were significantly greater in cancer patients than in normal subjects. Chowdhury et al. [39] explored the relation between polyamine spectrum and the degree of malignancy in human breast cancer patients. The results showed that polyamine levels varied with clinical staging of the disease and bore a direct relationship to the degree of the disease. Lee et al. [50] have suggested that the ratio of N^1-Acspm to Spd and estrogens 16a-OH E_1 to 2-OH E_1 may be considered as a useful dual marker for confirming the breast cancer. Kubo et al. [51] investigated polyamine metabolism in human heptacellular carcinoma for the malignancy and correlated ODC activity and the Spd to Spm ratio with tumor volume doubling time. Abdel-Monem et al. [52] have indicated that the ratio of N^1- to N^8-Acspd was higher in patients with non-Hodgkins lymphoma and elevated levels of N^1-Acspd in tissues and extra cellular fluids have been reported to be characteristic of malignant tumors

[53,54] but the valid use of the marker remains controversial [55,56].

4. Limitations as markers for cancer

Variation in the polyamine levels in diseases other than cancer have also been reported. Patients with cystic fibrosis [57], muscular dystrophy [58,59] psoriasis [60,61], hepatic failure [62], diabetics [63], rheumatoid arthitis [64] and uremic [65] indicate higher levels of polyamines in biological fluids. Increase in urinary polyamines has also been reported during normal pregnancy [66]. Polyamine synthesis could be stimulated in response to stressful condition such as electrical stimulation, traumatic injuries, neurotoxins and ischemia [67–72]. However, a high protein diet did not increase urinary polyamine excretion [73]. Loser et al. [17] observed a significant increase in polyamine levels in serum and urine of colorectal cancer as compared to healthy controls. However, nonmalignant gastrointestinal diseases partly showed similar tendency [74] (Tables 4 and 5).

Since polyamine concentrations in serum and urine normalized in patients after curative operations while these levels were further elevated in patients with proven tumor relapse and metastases, these substances may play a clinical role in predicting therapeutic success or indicating relapse of the tumor. A role of polyamines in carcinogensis is the observation that patients with elevated colon mucosal polyamines are at higher risk of colorectal cancer than people with normal concentrations [75].

5. Analytical procedures

Aliphatic diamines (Put and Cad), polyamines (Spd and Spm) and their acetyl conjugates do not contain a suitable chromophore or fluorophore group. Hence, they cannot be determined with adequate sensitivity by spectrophotometric or fluorescence detection. Therefore, most of the analytical procedures require different derivatizing reagents to increase the sensitivity of the method. The compounds have similar structural features, and for their selective separation they involved chromatographic [76], electrophoretic [77] radioimmunoassay [78] or enzymatic assay [79–81] procedures. The results of comparative sensitivities of common analytical techniques are summarized in Table 6.

In biological samples the polyamines are present in low concentrations (Table 1) and most of the derivatizing reagents react with amino acids which are present at higher concentrations. Therefore, effective separation methods are required for polyamine determinations in the presence of amino acids

Table 4
Total polyamine concentrations ($X \pm$S.E.M.) in the serum (nmol/ml) and urine (nmol/mg of creatinine), of parents with colon cancer, healthy volunteers, and patients with nonmalignant gastrointestinal and the corresponding sensitivity and speciality

		No.	Age (yr)	Serum (nmol/ml)				Urine (nmol/mg of creatinine)			
				PUT	CAD	SPD	SPM	PUT	CAD	SPD	SPM
Normal patients	$X \pm$S.E.M.	30	51.6	0.488	0.32	0.286	0.175	13.90	3.4	6.00	0.54
			1.8	0.03	0.07	0.01	0.01	1.0	0.5	0.5	0.08
Nonmalignant disease patients	$X \pm$S.E.M.	40	49.8	0.710	0.470	0.589	0.110	28.23	12.21	12.18	1.67
			1.7	0.08	0.05	0.06	0.02	2.5	1.8	0.9	0.2
Colon cancer patients	$X \pm$S.E.M.	50	58.1	0.765	0.380	0.640	0.180	37.3	15.7	20.62	1.48
			1.9	0.04	0.04	0.03	0.03	2.89	1.6	1.45	0.23
Significance III/I				0.01	NS	0.001	NS	0.001	0.001	0.001	0.005
Significance III/II				NS	NS	NS	NS	0.01	NS	0.005	NS
Sensitivity	(%)			60.25	50.50	89.15	34.30	84.00	66.3	0.92.1	74.3
Specificity	(%)			54.2	67.6	27.2	89.0	58.6	43.6	40.0	13.1

S.E.M.: Standard error of the mean; NS: not significant.
Data reproduced from Ref. [15], with permission.

Table 5
Free and acetylated polyamine concentrations ($X \pm$S.E.M.) in the urine (nmol/mg of creatinine) of patients with colon, cancer, healthy volunteers, and patients with nonmalignant gastrointestinal diseases, and the corresponding sensitivity and specificity

		No.	Urine (nmol/mg of creatinine)							
			AcPUT	PUT	CAD	N^1-Acspd	N^8-Acspd/SPD	N^1-Acspd/N^8-Acspd	SPD	SPM
Normal patients	$X \pm$S.E.M.	30	10.7 0.7	0.68 0.1	0.52 0.06	3.40 0.3	2.00 0.18	1.70 0.11	0.24 0.02	0.38 0.08
Nonmalignant disease patients	$X \pm$S.E.M.	40	21.75 2.2	2.19 0.17	1.96 0.17	6.52 0.57	4.87 0.43	1.69 0.19	0.76 0.07	1.04 0.16
Colon cancer patients	$X \pm$S.E.M.	50	31.93 2.7	2.75 0.33	2.03 0.28	11.92 0.7	6.28 0.48	2.29 0.13	0.72 0.09	0.65 0.11
Significance III/I			0.001	0.005	0.005	0.001	0.001	NS	0.01	NS
Significance III/II			0.05	NS	NS	0.05	NS	NS	NS	NS
Sensitivity	(%)		84.5	78.2	53.9	79.3	78.6	17.4	65.25	34.9
Specificity	(%)		53.6	50.5	40.4	65.5	61.5	92.2	30.0	44.5

S.E.M.: Standard error of mean.
Data reproduced from Ref. [15], with permission.

Table 6
Analytical methods used for polyamine assay

Method	Details	Relative sensitivity
Paper chromatography	Ninhydrin	0.1–0.5 μmol
Thin-layer chromatography (TLC)	Ninhydrin	5–100 nmol
	Dansyl derivatives	10–100 pmol
Overpressure TLC	Ninhydrin+Cd^{2+}	2–60 nmol
High-performance liquid chromatography (HPLC)	Tosyl derivatives	1–10 nmol
	Fluorescamine derivatives	50–20 pmol
	Enzymatic+chemiluminescent dansyl derivatives	5–500 pmol 10–100 pmol
Ion-exchange chromatography (amino analyzer)	Ninhydrin	1–50 pmol
	Fluorescamine	5–200 pmol
	Orthophthaldehyde	5–200 pmol
Gas chromatography	*N*-Trifluoroacetyl (*N*-TFA) derivatives	100–600 pmol
Electrophoresis	Ninhydrin	5–200 nmol
Mass spectrometry	*N*-TFA or dansyl derivatives	10–100 pmol
Gas chromatography–mass spectrometry	*N*-TFA derivatives+deuterated internal standards	1–10 pmol
Enzymatic methods	*Serrutia marcescens*	5–10 nmol
Immunonephelometry	Latex beads	1–10 pmol
Radioimmunoassay (RIA)	Purified/antibodies	50–200 nmol

Data reproduced from Ref. [76], with permission.

in biological samples based on pre- or post-derivatization with spectrophotometric, fluorometric or electrochemical detection. The chromatographic procedures have become attractive because of the developments in the chromatographic materials, detection devices and the possibilities that the methods could be automated. For the separation of the polyamines all types of chromatographic methods have been used mainly, liquid, gas and planar chromatography. Recent advances in the field of capillary electrophoresis (CE) [82] has enabled use of CE for the determination of polyamines.

5.1. Planar chromatography

Paper chromatography (PC) is one of the oldest methods for the analysis of polyamines where ninhydrin has been used as a locating reagent. Semi quantitative assay is possible after extraction of the ninhydrin spots by 75% ethanol [83]. Wiesner [84] reported R_F values for 19 polyamines and three monoamines using PC. The method is rarely reported in recent literature and has been replaced by thin-layer chromatography (TLC).

TLC is the simplest analytical technique. Cellulose or silicic acid plates are used to analyse free polyamines or their coloured or fluorescent derivatives [85,86]. TLC methods for assaying polyamines have been recently reviewed [87]. The commercialization of high-performance thin-layer plates (HPTP) (Merck, Whatman) has contributed towards excellent separations of amines by this method specially when bidimensional techniques are employed [88].

Bachrach [83] has summarized the use of common solvents for the evaluation of R_F of the free polyamines. Ninhydrin has been used as locating reagent. The stained spots can be scrapped off the plate and extracted with ethanol for quantitation at 570 nm. Quantitation is also possible with the help of a scanner after spraying the chromatograms with suitable derivatizing reagent. Abe and Samejina [89] used fluorescamine to give fluorescent derivatives with increased sensitivity. Blankenship and Walle [90] separated two isomeric monoacetylated (N^1 and N^8) Spd on silica gel plates using a solvent system of chloroform–methanol–ammonium hydroxide (2:2:1) for the separation of N^1-Acspd and N^8-Acspd giving an R_F of 0.37 and 0.47, respectively. Seiler and Knodgen [91] determined Put, Spd and Spm as dansyl derivatives from mouse liver tissue, using 5×5 cm HPTPs developed with a solvent mixture of cyclohexane–ethyl acetate (1:1). Abdel-Monem et al. [88] have used two-dimensional TLC (Silica gel GF 250 μm) to analyse the free and acetylated polyamines in human urine. Cyclohexane–diethyl ether (1:9) was used as a solvent for the first dimension and chloroform–triethylamine (10:1) in the second dimension to chromatograph the dansylated derivatives. Kramer and Unger [92] determined agmatine, tyramine, histamine, Cad and Put by using ion-exchange TLC on Fixion 50×8 chromato sheets, developed in a NaCl–sodium citrate buffer (pH 6) containing 2.5 *M* Na^+. A telechrom S type automatic video-densitometer was used for quantitative evaluation. Wettlaufer and Weinslein [93] separated dansylated polyamines by TLC on Whatman LK6D 20×20 cm glass plates using triethylamine–chloroform (4:25) or ethyl acetate–cyclohexane (5:4). The dansylated polyamines were than determined from the plates using a UV fluorescence video scanner and image analyzer. Chowdhury et al. [39] have estimated the role of polyamines by TLC method for the management of breast cancer.

Over-pressurized thin-layer chromatography (OPTLC) has been developed and commercialized [94,95]. Bardocz et al. [96] used the Chrompress 25 to separate ornithine, arginine, Put, Spd and Spm in 30 min using Fixion 50×8 cation-exchange TLC sheets which were eluted by 300 m*M* potassium phosphate buffer (pH 7.5) containing 2 *M* NaCl. The method was used to determine the polyamines in crude extract of liver, kidney, lungs and thymus of mouse.

5.2. Gas chromatography

The use of GC for the analysis of polyamines in biological samples is gradually increasing. Enormous abilities of high-resolution capillary column GC together with selective detection systems have enabled GC for quantitation of polyamines at high sensitivity and selectivity [97,98]. GC of the polyamines without derivatization is possible [99], but derivatization with suitable reagents improves GC elution and resolution with considerable enhancement in the sensitivity of the detection system [43].

Bakowski et al. [43] analysed Spd, Spm and Put as isobutyloxycarbonyl derivatives on a mixed phase of 1.5% SE-30, 0.15% FF APon Chromosorb WHP, using an N-sensitive glass bed detector and helium as a carrier gas. The method was applied for the determination of plasma polyamines and its application in tumor has been shown. Battenbury et al. [100] determined urinary di- and polyamines after acid hydrolysis. Pentafluoropropionyl derivatives were analysed by GC coupled with electron-capture detection (ECD). Programmed temperature elution was carried out from 130 to 250°C at 8°C/min from 3% OV-255 on a Ga Chrom Q column with $Ar–CH_4$ (95:5) as carrier gas and the method of quantitation for polyamines were made at pmol levels. Jiang [101] determined polyamines in urine of normal human and cancer patients by capillary GC after derivatization with trifluoroacetic anhydride. Recoveries of Put, Spd and Spm of 96.7, 102.6 and 98.7%, respectively, were reported with a standard deviation of 1.949 ± 0.041 μg/mg creatinine (Tables 1 and 2). Suh and co-workers [15,40] determined urinary free and acetylated polyamines (five free and four acetylated) with capillary GC with nitrogen–phosphorous detection. Derivatization was carried out with heptafluorobutyric anhydride and recovery ranged between 48.6 and 101.2%. Capillary GC based on derivatization with trifluoroacetylacetone has also been used for the determination of Put and Cad from serum and urine of cancer patients, before and after radiotherapy. Flame ionization detection (FID) was used giving detection limits of 0.5–0.6 ng/injection. Similar method of analysis was used to quantitate Put and Cad in the serum of two healthy persons [102,103]. Muskiet et al. [104] have reviewed HPLC and GC profiling methods for the quantitation of polyamines, polyamine conjugates and their metabolites in tissues, cell and extracellular fluids.

5.3. Liquid chromatography

Among the analytical methods for the determination of the diamines, polyamines and their conjugates, more procedures are available based on high-performance liquid chromatography (HPLC). For detection purposes, spectrophotometric and spectrofluorimetric detection devices are frequently used, followed by electrochemical or enzymatic post-column detection. The common derivatizing reagents reported for the purpose are ninhydrin [105], ortho-phthaldehyde (OPA) [106], *N*-succinimidyl-3-ferrocenylpropionate [107], 9-fluoroenylmethyl chloroformate (FMOC) [108], benzoyl chloride [109,111], 3,5-dinitrobenzyl chloride [112], dansyl chloride [113–115], acetylacetone [116,117], 8-quinoline sulfonic acid [118], fluorescamine [119], 2-(1-pyrenyl)ethylchloroformate (PEOC) [120], 2-hydroxynaphthaldehyde [121], carbazole-9-ylpropionic acid [122], *N*-hydroxysuccinimidyl-6-quinolinyl carbamate [123], 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [124], 2-(9-anthryl)ethylchloroformate [125], 4-(*N′*-phthalimidyl) benzene sulfonyl chloride [126] dabsyl chloride [127], 4-(2′-phthalimidyl)benzoyl chloride [128], 1-phenylsulfonyl-3,3,3-trifluoropropene [129], 1-naphthylacetic anhydride [130] and 2-chloroethylnitrourea [131].

The procedures are based on the separations of the polyamines from ion-exchange or ion-pair chromatography, followed by postcolumn derivatization with suitable reagent. Alternatively the polyamine derivatives formed are separated on a reversed-phase or an ion-pair reversed-phase column using isocratic or gradient elution procedures.

5.3.1. Postcolumn derivatization

Fully automated instruments are available from different sources, which reduces manual labour, and variability in the results. The commercial availability of special beds of sulfonated cross-linked styrene–divinylbenzone copolymer with narrow ranges of diameter 10 μm, 5 μm or less has improved the resolution and the sensitivity of the method.

Sensitivity of the method depends on the reagents used (Table 6). Ninhydrin allows nmolar determination by photocolorimetry, while fluorimetry using fluorescamine or OPA allows picomolar determinations. Among the postcolumn derivatization procedures OPA is the most reported reagent.

Marton and Lee [132] used OPA in place of ninhydrin to derivatize primary amines in automated HPLC for the determination of polyamines. A 1–10-fold increase in sensitivity was reported as compared to ninhydrin. The method was applied for the analysis of physiological fluids and tissue extracts.

Villanueva and Adlakha [133] developed an auto-

mated separation of common basic amino acids and mono-, di- and polyamines as well as phenolic and indoleamines. The pmol level determination was carried out by ion-exchange column chromatography on a single sample in 160 min based on postcolumn derivatization with OPA. Three sodium citrate buffers were used for elution. Recovery after column chromatography was reported in the range of 43.2 to 97.3%. The method was examined for its application to crude samples such as microorganisms, vegetables, platelets and urine (Fig. 4).

Nakamura et al. [134] separated dipeptides, polyamines and related amino acids on a micro particulate strong cation-exchange resin (Particil 10Scx) with lithium citrate buffers and in stream fluorometric detection with OPA.

Villanueva et al. [135], have subsequently reported separation of natural polyamines and basic amino acids on HC-X7 cation-exchange resin Li^+ in 110 min. Two lithium citrate buffers were used for elution. The separation was also performed on DC 4A cation-exchange resin equipped with fluorometer using two sodium citrate buffers [136]. Urinary, plasma and erythrocytes polyamines were quantitated using different composition of sodium citrate buffers [137–139]. Villanueva [76] in his recent review described their two procedures using the amino acid analyser. The method of analysis has been adopted routinely for the analysis of crude samples, from many biological sources, but the major disadvantage of their method is the high cost of the instrument and its maintenance.

Seilar and Knodgen [140] reported the separation of natural polyamines and their monoacetyl derivatives by reversed-phase HPLC. Octane sulfonate was used to form an ion-pair with polycations and the OPA method was used for the postcolumn derivatization. The method allowed polyamine and Acspd determination directly from tissue extract and body fluids. They further improved the separation with octane sulfonic acid as ion pairs of the natural di- and polyamines and related compounds by em-

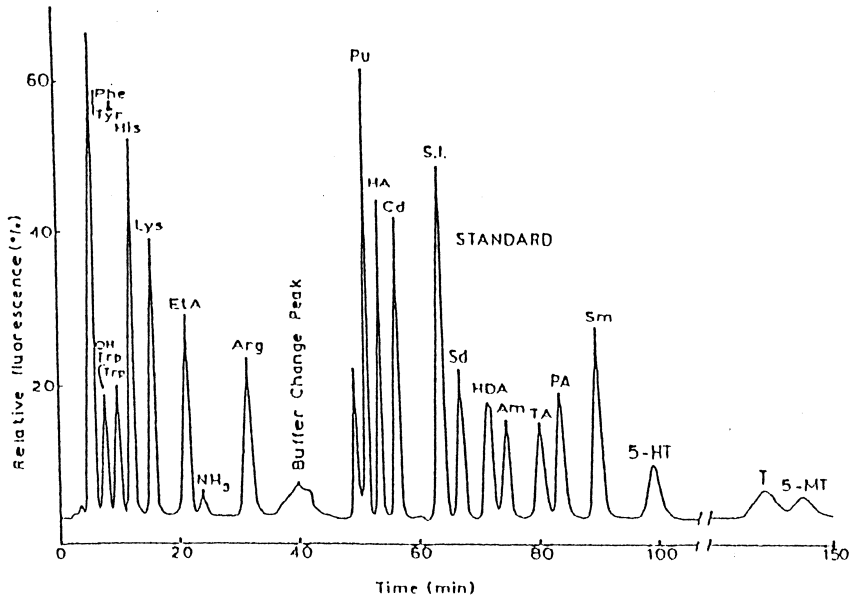

Fig. 4. Chromatogram of standard mixture, all at 500 pmol except for internal standard 375 pmol, compounds OHTtrp=5-hydroxy-tryptophane, TrP=trypophane, His=histidine, Lys=lysine, EtA=ethanolamine, NH_3=ammonia, Arg=arginine, DAP=1,3-diaminopropane, Pu=putrescine, HA=histamine, Cd=cadaverine, 1.S=4-azaheptamethylenediamine, Sd=spermidine, HDA=hexamethylane-diamine, Am=agmatine, TA=tyramine, PA=phenylethylamine, Sm=spermine, 5-HT=serotonine, T=tryptamine, 5-MT=5-methoxy-tryptamine. Gradient elution with three sodium citrate buffer systems from a 9.5×0.45 cm column of DC-4A, Na^+ cation-exchange resin, with postcolumn derivatization with OPA–2-mercaptoethanol. Detection: fluorometric. From Ref. [76], with permission.

ploying a column with high capacity factor and gradient elution with a three-solvent system. The separation system was applied to the analysis of urine samples and tissue extract [98] (Fig. 5).

Gamoh and Fujita [141] separated Put, Cad, Spd, and Spm as ion pairs of sodium hexane sulfonate on a reversed-phase column. Loser et al. [142] described a method for determination of di and polyamines together with their monoacetyl derivatives in biological samples. Ion pair reversed-phase HPLC was used after urine and serum samples purified on Bond Elut silica cartridge. The detection limit for polyamines was 0.5–1.0 pmol. Izquierdo-Pulido et al. [143] determined biogenic amines by ion-pair LC on a column of Nova-Pak C_{18} with gradient elution (1 ml/min) and postcolumn derivatization with OPA. The detection limits ranged 0.3–0.65 mg/l.

Seiler and Knodgen [18,140] methods have been applied to the determination of polyamines in mouse fibroblasts and mouse leukemia cells [144], human colon tumor cells [145], control and resistant L1210 cells to difluoromethylornithine [146], polyamine transport system in Chinese hamster ovary cells [147], tumor growth rate of Lewis lungs carcinoma [148], characterization of a diamine exporter in Chinese hamster ovary cells and identification of specific polyamines substrate [149], hormonal and feedback regulation of Put and Spd transport in

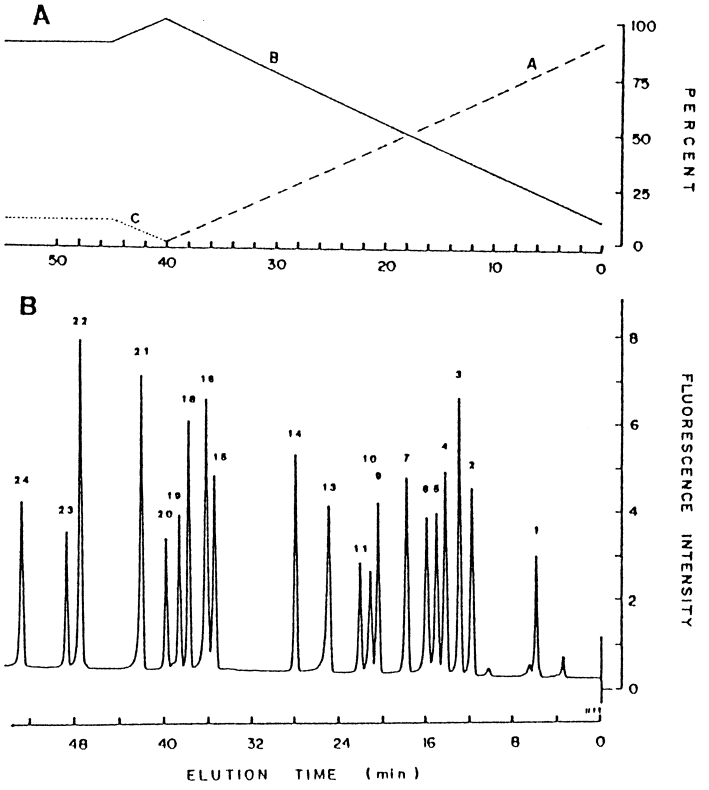

Fig. 5. Separation of polyamines and related compounds using gradient of (A) 0.1 *M* sodium acetate (pH 4.5) with sodium octane sulfonate (10 m*M*), (B) 0.2 *M* sodium acetate (pH 4.5)–acetonitrile (10:3) with sodium octane sulfonate (10 m*M*) and (C) methanol. (A) Composition of gradient. (B) Fluorescence intensity after reaction with OPA–2-mercaptoethanol. Amount of each compound is 1 nmol per 0.1 ml. Compounds: (1) tyrosine, (2) anserine, (3) Phenylalanine, (4) carnosine, (5) homocarnosine, (6) Acput, (7) putreanine, (8) *S*-adenosylmethionine, (9) arginine, (10) Accad, (11) trytophan, (12) 5-methylthioadenosine, (13) isoputreanine lactam, (14) isoputreanine, (15) put, (16) cad, (17) decarboxylated *S*-adenosylmethionine, (18) histamine, (19), N^1-Acspd, (20) N^8-Acspd, (21) 1,7-diaminoheptane (internal standard), (22) Spd, (23) N^1-Acspm, (24) spm. From Ref. [18], with permission.

human breast cancer cells [150] and Put accumulation and eukaryotic initiation factor 5A (IF-5A) [151]. The Loser et al. [142] method was used for determination of polyamines in colorectal cancer [17].

5.3.2. Postcolumn enzymatic reaction

Maruta et al. [152] and Watanabe et al. [153] separated di- and polyamines by isocratic ion pairing reversed-phase chromatography. The separated di- and polyamines were enzymatically converted with the release of H_2O_2, via postcolumn reaction with immobilized enzyme polyamine oxidase. H_2O_2 was detected by electrochemical oxidation on a platinum electrode. The detection limit of injected di- and polyamines were reported in the range of 0.3–4 pmol, with linear range of 2–3 orders of magnitude. The H_2O_2 produced by this procedure was also quantitated with chemiluminescent detection [154]. Hiramatsu et al. [155] developed an improved system for simultaneous measurement of nine free and acetylated polyamines. The separated polyamines were introduced with the enzyme reactor, in which these deacetylated were oxidized to generate H_2O_2 which was detected by electrochemical detection. The method was later extended for detection of diacetylpolyamines namely N^1,N^8-diacetylspermidine (N^1,N^8-Dacspd) and N^1,N^{12}-diacetylsperimine (N^1N^{12}-Dacspm) with detection limits between 0.6 and 0.9 pmol [56] (Table 2). The methods were applied for the determination of polyamines in urine and rat brain homogenates.

5.3.3. Precolumn derivatization

Ion-exchange or ion-pair reversed-phase chromatography with postcolumn derivatization have been used with adequate sensitivity however, to improve the sensitivity further with the use of less amount of expensive derivatization reagent, and achieve short analysis time, precolumn derivatization procedures are preferred. Among the precolumn derivatizing reagents reported for the determination of polyamines, OPA, fluorescamine, dansyl chloride, benzoyl chloride, FMOC and related compounds are frequently reported.

5.3.3.1. Orthophthaldehyde. OPA in the presence of 2-mercaptoethanol is a selective reagent reacting only with primary amino groups. However, the derivatives formed are unstable, especially for Spd and Spm due to presence of secondary amino groups.

Mell Jr. [156] separated and determined polyamines by reversed-phase chromatography (μBondapak column) after precolumn derivatization with OPA using gradient elution program, followed by fluorometric detection. The detection limit of 100 pg injected on the column with linear response up to >30 ng was reported. Polyamines after sample clean up on a disposable CM-cellulose column, were determined as OPA derivatives in tissue and body fluids on a C_{18} column in isocratic mode with 0.02 *M* borate buffer–0.15 *M* NaCl (pH 9) with analysis time 12–15 min/sample was achieved [157]. Quantitation of ODC was reported by the precolumn assay of Put with OPA on a C_{18} column. Elution was made with methanol–0.05 *M* sodium acetate buffer, pH 5.9 (70.5:29.5, v/v) [158]. A modified method was also reported to quantify polyamines from brain tissue (>0.3 mg) which were extracted by perchloric acid and OPA derivatives separated on a reversed-phase column with gradient elution [159]. Instead of fluorescence detection polyamines were monitored as OPA-thiol derivatives electrochemically at 0.65 V, using 0.1 *M* perchloric acid/*N*,*N*-dimethylcyclohexylamine and methanol (80%) as eluting buffer. A gradient of 60 to 90% methanol gave improved separation, with linear response from 1 pmol to 10 nmol [160]. Others have also reported HPLC separation of polyamine-OPA derivatives with electrochemical detection [161–165]. Saito et al. [166] developed a method based on column derivatization of polyamine with OPA and *N*-acetyl-L-cysteine and elution was made on a C_{18} polymer column using acetonitrile and alkali borate buffer. The application of the method resulted in 85% recovery of polyamines from food samples. Automated method with the reversed-phase material Inertsil was used to separate OPA derivatives of amino acids, from OPA derivatives of di- and polyamines [167]. A similar procedure was reported using a Shodex Rspak DE 613 column with gradient elution and on column derivatization with OPA–*N*-acetylcystine [168].

5.3.3.2. Fluorescamine. A method of analysis based on precolumn derivatization of polyamine with fluorescamine was developed. In this method the

sample of rat liver, human serum and urine after pretreatment with perchloric acid was introduced to CM-cellulose column chromatography in pyridine–acetic acid buffer. The evaporated fractions were dissolved in borate buffer and reacted with fluorescamine. Aliquots of reaction mixture were subjected to HPLC separation with fluorometric detection. The method was used to assay biological samples containing less than 100 pmol of amines with 5% RSD [169]. A method where the presence of nickel ions in the precolumn derivatization procedure was used, which inhibited the interfering amines with fluorescamine. The derivatives were separated on LiChrosorb RP-18 with linear gradient elution with detection limits within the range of 5–10 pmol of amines [119]. The method was later automated [170].

5.3.3.3. Benzoyl chloride. HPLC methods of analysis based on benzoylated derivatives have been reviewed recently [171]. In the process of method development based on the reagent, polyamines were determined by benzoylation and reversed-phase HPLC on Dpher RP-8 with 40% aqueous methanol as mobile phase. The sensitivity limit was 100 pmol/ml [172]. Another method of analysis was described for the quantitation of di- and polyamines and acetylated derivatives of Spd and Spm in biological fluids with either benzoyl chloride or 3,5-dinitrobenzoyl chloride. High sensitivity was achieved with 3,5-dinitrobenzoyl chloride [112]. An improved method based on derivatives of benzoylated polyamines in aqueous methanol solution was reported on a reversed-phase C_{18} column. The minimum detection concentration of 0.25 nmol/l was reported [173]. Attractive HPLC methods were described for the assay of polyamines and their monoacetyl derivatives with UV detection giving a linear relationship between 1.25 and 25 nmol/ml [109,110] (Fig. 6). Watmabe et al. investigated the interfering products in HPLC determination of polyamines as benzoyl derivatives. They observed a change in the size of two peaks with the reaction time of benzoylation and the standing time of the benzoyl polyamines in methanol before the separation procedure [174]. Mono-, di- and polyamines were derivatized with benzoyl chloride and separated on a LiChrospher 100 RP-18 column using a gradient elution system with a methanol water in 10 min [175]. Reversed-phase HPLC was reported for the determination of eight polyamines and monoacetylpolyamines. The interfering products were identified and eliminated and the method was applied to determine polyamine contents in P 388 Cancer cells [176]. Benzoyl chloride derivatives of Put, Spd and Spm were also separated on a μBondaPak C_{18} reversed-phase column with 50% methanol as the mobile phase. The method was applied to the analysis of polyamines in blood serum [41]. Polyamines were extracted with 5% perchloric acid and detected at 254 nm. Linear calibrations were reported in the range 0.2–3.4 μmol [177]. Benzoylated Put, Cad, norspermidine, Spd, norspermine and Spm were separated on a reversed-phase column using acetonitrile–water (42:58) as mobile phase and UV detection at 198 nm. The detection limits were in the range of 0.8 to 1.3 pmol [178]. Normal and mifestone treated pregnant rat uterus, with the embryonic tissues were homogenized with 10% trichloroacetic acid. The supernatant was derivatized with benzoyl chloride and separated and determined by RP-HPLC after repeated washout by 0.1 mol/l NaOH to avoid interference [179]. Put, Spd and Spm were derivatized by reaction of 4-(2-phthalimindyl)benzoyl chloride in the presence of $NaHCO_3$ and the product thus obtained were separated on an ODS column and then determined by RP-HPLC with a detection limit of 0.1 pmol [128].

5.3.3.4. Dansyl chloride. Dansyl chloride (5-dimethylaminonapthalene-1-sulfonyl chloride) reacts with primary as well secondary amino groups with the result that Put, Spd and Spm form fluorescent di-, tri- and tetradansyl derivatives, respectively [180,181]. HPLC methods for the determination of polyamines using dansyl chloride have been reviewed [182]. Put, 1,6-diaminohexane, Spd and Spm derivatives separated on a μBondapak C_{18} column with 1-heptanesulfonic acid and acetonitrile as mobile phases, using programmed solvent gradient system separating within 30 min giving detection limit of 1 pmol [183]. Using normal-phase, di- and polyamines after derivatization were also reported using a Silica 60 HPLC 5 μm, C_{18} column with a gradient of 10%, acetonitrile in 0.02 *M* acetic acid to a 1:1 mixture of methanol and 10% 0.02 *M* acetic acid in acetonitrile. The amines were detected at 254

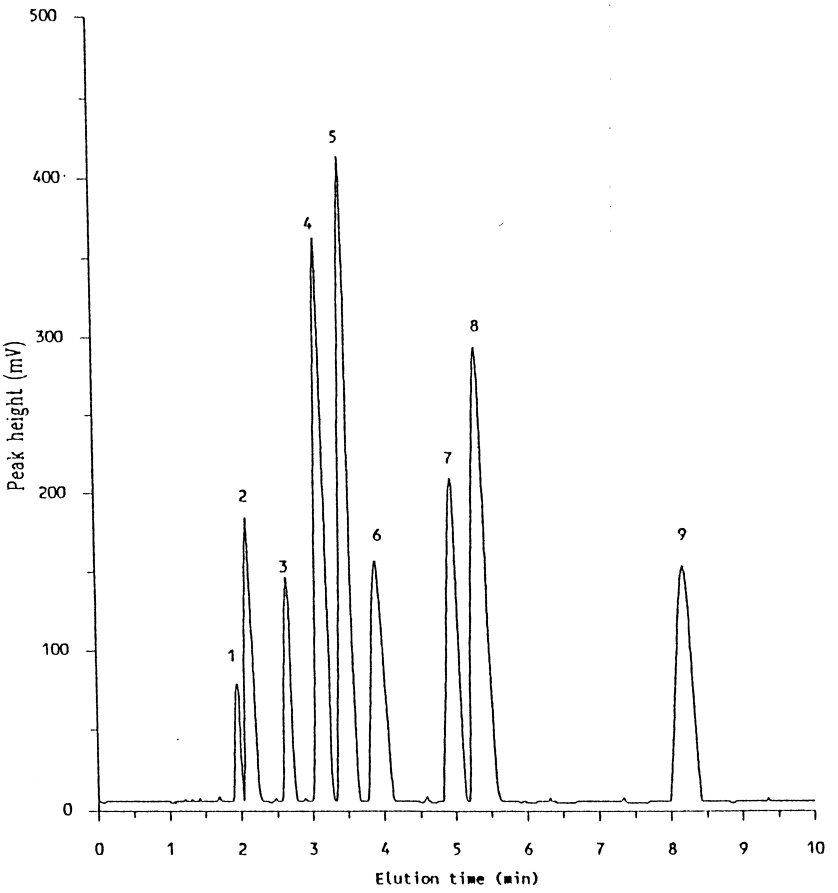

Fig. 6. HPLC elution profile of benzoylated polyamines and their monoacetylated forms (standard mixture) amount of each compound 0.25 nmol/0.020 ml. Peaks: (1) *N*-Acput, (2) *N*-accad, (3) N^1-acspd, (4) Put, (5) Cad, (6) N^1-acspm, (7) Spd, (8) 1,7-diaminoheptane (internal standard), (9) Spm. From Ref. [109], with permission.

nm [184]. Another analysis method was reported where the procedure was adopted in which after deproteinization, polyamines were dansylated and extracted on a waters C_{18} Sep-Pak cartridge. The samples were analysed by HPLC using a step solvent change and a 3 μm C_{18} column. The separation of three polyamines was achieved within 10 min [113]. In plant samples, di- and polyamines were extracted by an organic solvent prior treatment with dansyl chloride. The excess dansyl chloride was masked with proline and polyamines analysed on an ODS Hypersil column with a programmed water methanol changing from 60 to 95% in 23 min with fluorescence detection at excitation and emission wavelengths of 365 and 510 nm, respectively [185]. After derivatization with dansyl chloride, unconjugated polyamines and acetylated polyamines in biological fluids were extracted by elution from a Bond Elut C_{18} column and then separated on a reversed-phase column with gradient elution. The analysis time of 40 min showed complete separation of unconjugated and monoacetylpolyamines [114]. Rat brain and other tissues samples were processed and derivatization was performed overnight at ambient temperature. After the derivatives extracted by benzene, the separation was achieved on a LiChrosorb RP-18 column using a gradient of acetonitrile–1.2 m*M* Na_2HPO_4 (8:2) in 1.2 m*M* Na_2HPO_4 [186].

Various newly developed HPLC methods were introduced with some modification where Put, Spd, Spm and their conjugated metabolites were separated [187–194]. Among these methods of analysis, the

separation and quantitation of dansylated polyamines was reported using a 33×4.6 mm I.D., 3 μm particle size C_{18} cartridge column and a linear gradient of acetonitrile–heptane sulfonate (100 m*M*, pH 3.4) at a flow-rate of 2.5 ml/min giving complete separation in less than 8 min with detection limits of 1 pmol per 6 μl for each polyamine [191]. A complete separation of 42 amines is shown in Fig. 7 [194]. In urine sample from cancer patients, determination of polyamines was worked out by hydrolysis, derivatization and extraction of clinical samples. Reversed-phase HPLC with gradient elution of water organic mobile phase was carried out using UV and fluorescent detection [195]. The separation of di- and poly-

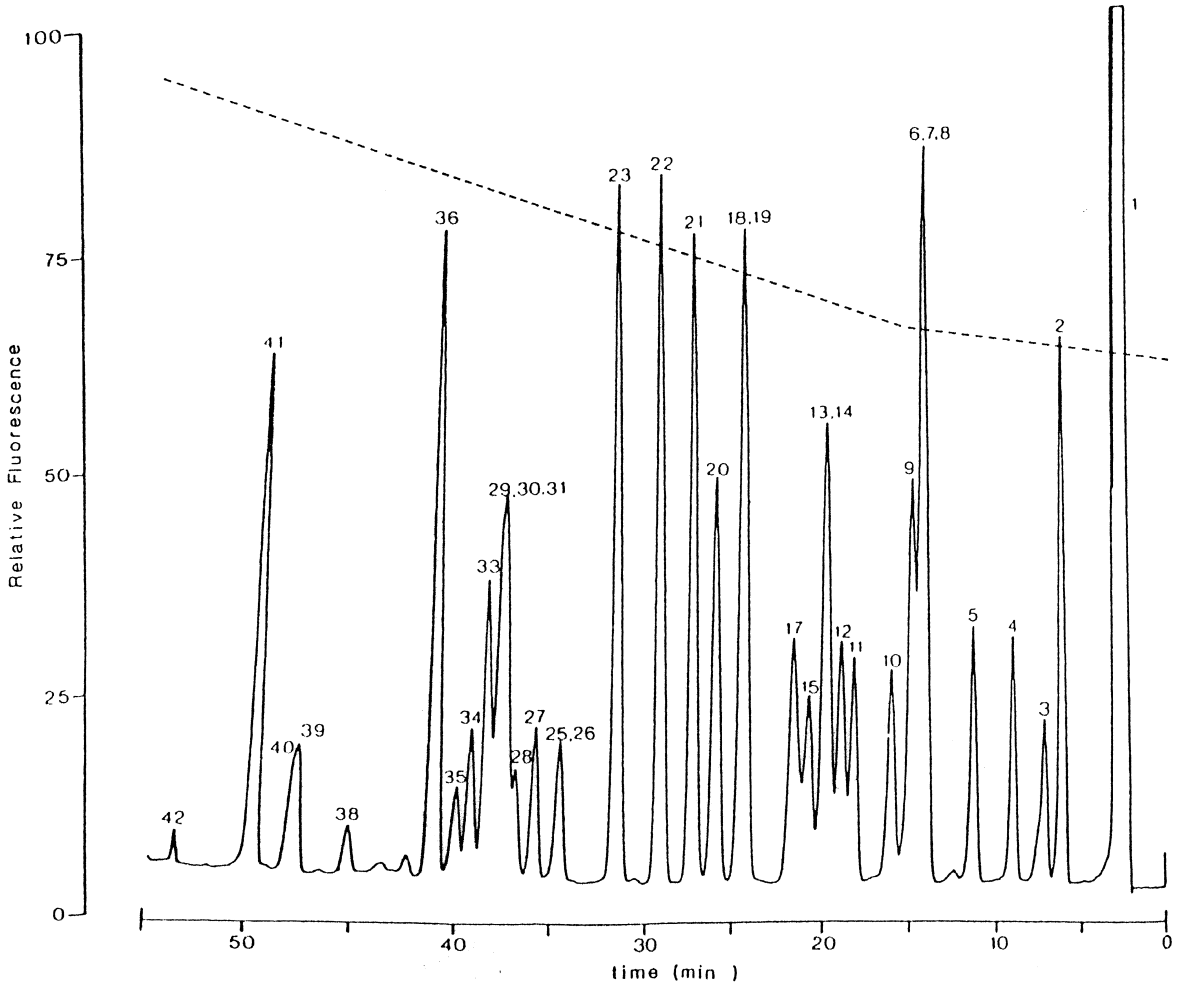

Fig. 7. HPLC of dansylated standards separated on a Spheri-5RP-18, 5 μm reversed-phase column. Methanol–water gradient elution with flow-rate 1 ml/min and fluorometric detection at excitation 340 nm and emission 540 nm. Each peak represents 0.9 nmol of amine. Peaks: (1) dansyl-OH, (2) ammonia, (3) ethanolamine, (4) methylamine, (5) ethylamine, (6) dimethylamine, (7) isopropylamine, (8) dansyl-Cl, (9) *n*-propylamine, (10) phenylethanolamine, (11) norephedrine, (12) isobutylamine, (13) *n*-butylamine, (14) benzylamine, (15) tryptamine, (16) agmatine, (17) L-ephedrine, (18) isoamylamine, (19) 2-phenylethylamine, (20) 1,3-diaminopropane, (21) Put, (22) Cad, (23) 1,6-diamino-hexane, (24) *N*-methyl-putrescine, (25) DL-octopamine, (26) histamine, (27) 3-methyoxy-4-hydroxybenzylamine, (28) serotonin, (29) metanephrine, (30) 3-hydroxy-4-methoxyphenylethylamine, (31) 3-methyoxy-*p*-tryamine, (32) 1,7-diaminoheptane, (33) DL-synephrine, (34) *p*-tyramine, (35) *o*-tyramine, (36) Spd, (37) homospermidine, (38) norepinephrine (39) DL-epinephrine, (40) dopamine, (41) Spm, (42) 5-hydroxydopamine. From Ref. [194], with permission.

amines as their dansyl derivatives, on a reversed-phase column using a mobile phase of acetonitrile–water [196] or methanol–water [197] was also achieved. The recovery was reported >97%. The detection limits for Put, Spd and Spm were 0.05, 0.08, 0.06 nmol/ml, respectively [197].

Free polyamines and their acetylated derivatives were also separated using ion-pair reversed-phase HPLC after precolumn derivatization with dansyl chloride. Detection limits were reported between 2.27 and 33.6 fmol/μl [198].

In spite of the fact the reaction time of dansyl chloride at ambient temperature takes longer time (overnight), which could be reduced significantly by warming at higher temperature, however, multiple derivatives can be formed and the excess of the reagent may interfere the analyte signal [186,192]. Despite the mentioned disadvantages, dansyl chloride is still used extensively for the quantitation of polyamines in biological samples. Among its clinical applications, it has been used for the determination of polyamines in cell culture media [199], high-salt solution [200], sausages [201], intracellular amines in tumor cells [202], tumor promotion in mouse skin [203], during growth inhibition following polyamines depletion [204], blood polyamines in rats [205], polyamines in MCF-7 breast cancer cells [206], polyamine pool depletion and growth inhibition in human melanoma cell lines [207], polyamines in duodenal mucosal repair after stress in rats [8], polyamine concentrations in human digestive lumen [21], polyamines in a cabus opella primates model [208] and in resistant human overian carcinoma cells [10,209].

5.3.3.5. 9-Fluorenylmethyl chloroformate and its analogs. 9-Fluorenylmethyl chloroformate (FMOC-Cl) and its analogs are used in the precolumn derivatization of amino acids and polyamines. These reagents react with primary and secondary amino groups forming fluorescent derivatives in buffered aqueous solution.

The determination of polyamines consisted the formation of FMOC adducts, followed by stepwise elution, utilizing a NovaPak C_{18} column, methanol–water (82:18) then by 100% methanol and fluorometric detection (excitation at 260 nm, emission at 313 nm). Put, Cad, Spd, Spm separated within 13 min with detection limits in the range 22.7 to 109 fmol [210]. The method has been applied to the determination of polyamines concentration in rat dorsal root ganglia, and in cerebrospinal fluid of patients with brain tumor without interference with endogeneous amino acids [211,212]. Gilbert et al., described a clean up procedure for the isolation of basic amino acids and polyamines using a weakly acidic cation-exchange resin, Biorex-70 (Bio-Rad), followed by derivatization, with FMOC and separation by a reversed-phase HPLC [108]. Wickstroen and Betner [213] developed a separation method for acetylated and monoacetylated polyamines, based on precolumn derivatization with FMOC on a reversed-phase column. The derivatization reaction was completed in 2 min and excess FMOC was derivatized with glycine, which reduced interferences from untreated reagent to minimum. Huhn et al. [214] described the method for di- and polyamines on a reversed-phase C_8 column with a linear gradient of acetate buffer–acetonitrile as mobile phase after derivatization with FMOC within 3 min at pH 7.8, in the presence of aspartic acid to remove excess reagent. The FMOC polyamine derivatives were reported stable in methanol–water for 10 h. Bellagamba et al. [215] reported HPLC determinations of polyamines in milk based on FMOC-derivatization and a gradient elution programme of water–acetonitrile with a linear curve in the range of 0.5–5 nmol/ml. Limits of detection calculated on the basis of three times the signal-to-noise ratio was 50 pmol/ml for each polyamine. Bauza et al. [216] reported determination of biogenic amines, di- and polyamines in urine samples, based on FMOC derivatization and separation was performed on a 5 μm Hypersil ODS column and complete separation was achieved in 110 min.

Other similar reagents such as 2-(9-anthryl)-ethylchloroformate (AEOC) and (1-pyrenyl)-ethylchloroformate (PEOC) were also used in the precolumn derivatizing mode for quantitation of polyamines: Put, Cad, Spd and Spm in serum samples from healthy individuals and cancer patients [120,125]. The detection limits reported were more favourable than polyamines derivatized with FMOC. Generally, FMOC methods suffer from the disadvantage that an excess of strong fluorescent reagent has to be extracted with pentane in order to stop the

derivatization reaction and to avoid spontaneous hydrolysis of FMOC adducts. The second widely used method to remove the excess FMOC after the derivatization process is by addition of amino acids, aspartic acid or asparagine [214,217].

5.3.3.6. Other reagents. 6-Aminoquinoyl-*N*-hydroxy succinimidyl carbamate [124] and *N*-hydroxysuccinimidyl-6-quinolinyl carbamate (HSQC) [123] have been reported for reversed-phase HPLC determination of polyamines and their acetyl conjugates. After derivatization, elution was carried out on 5 μm, C_8 or C_{18} reversed-phase columns with gradient elution with three-solvent systems and the quantitation is made by fluorescence detection. This method of derivatization provides with stable derivatives, reproducible results and a linear relationship between the fluorescence yield and concentration from 30 to 680 fmol (injection volume 10 μl) which shows the method being more sensitive than those with OPA and dansyl chloride. The method is applied to biological samples for the analysis of polyamines in extracts of mouse erythrocytes, trypanozoma, brucei and homogenates of pancreatic cancer Xeno-grafts.

You et al. [122] described the use of carbazole-9-ylpropionyl chloride (CRP-Cl) as derivatizing reagent for the separation of polyamines and amino acids in precolumn derivatization mode on a C_{18} column using a binary gradient elution. Primary and secondary amines reacted with CRP-Cl in alkaline medium. The separation of polyamines extracted from plant tissue was within 25 min. Linearity for the amount of polyamines injected was in the range 50–250 pmol with RSD<5%. However, the presence of different halide salts, organic solvents and surfactants affected the fluorescence intensity.

A few reagents such as 2-hydroxynaphthaldehyde (HN) [121], acetylacetone [116,117] and benzoylacetone (unpublished work) were also introduced as precolumn derivatization reagents.

5.3.4. Capillary electrophoresis

Capillary electrophoresis has developed enormously during the last decade in terms of analytical technique, detection devices, sample introduction techniques and application to the analysis of biological samples. Fast analytical scale separations by capillary electrophoresis have been reviewed [82]. Zhou et al. [218] described a method for the determination of polyamines in serum by capillary zone electrophoresis (CZE) with indirect ultraviolet (UV) detection. The concentrations of polyamines in serum of six healthy adults were determined and the results obtained were similar to HPLC. However, the author claimed that CZE was superior to HPLC in terms of sensitivity and small sample size. Mattausch et al. [219] applied CZE for the separation and quantitation of Put, Cad, Spd and Spm after their derivatization with fluorescin isothiocyanate. The derivatization and operation conditions were optimized with respect to laser-induced fluorescence detection (excitation 488, emission 520 nm). The detection limits were within 0.7–3.2 nmol/l with RSDs of 4.6 to 12%. Ma et al. [220] separated polyamines and some related amino acids by CZE with indirect photometric detection using 60 cm×75 μm I.D fused-silica and quinine sulfate as background electrolyte in 10 min. The fmol amounts of polyamines extracted from tumor cells were detected from a nl injection volume. Zhang and Yeung [221] described the construction and utility of a postcolumn reactor using OPA–2-mercaptoethanol as the fluorescent labelling reagent with laser-induced fluorescent detection. Mass limits of detection were in the low-amol range for various amines, amino acids and proteins. Oguri et al. [222] separated histamine, tyramine, Cad and Spd and detected at 340 nm by using 2 m*M* OPA–*N*-acetylcystine. A 20 m*M* sodium dodecyl sulfate (SDS)–20 m*M* phosphate–borate buffer (pH 10) was used as a run buffer at an applied voltage of 25 kV. Legaz et al. [223] reported the separation of tosylated Put, Cad, Spd and Spm by CZE in an uncoated fused-silica capillary. The optimal conditions for separations between Put and Cad were 5 m*M* sodium phosphate buffer, pH 2.2 with a gradient voltage that started at 10 kV and then jumped to 30 kV in different steps. Kovacs et al. [224] reported a capillary electrophoresis method for the determination of seven biogenic amines in food staff. The derivatization step with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate required sample preparation. Separation was achieved in 30 min (Fig. 8).

Capillary electrophoresis indicated better detection limits than GC or HPLC for polyamines analysis, but RSD is slightly on higher side. However, there is a

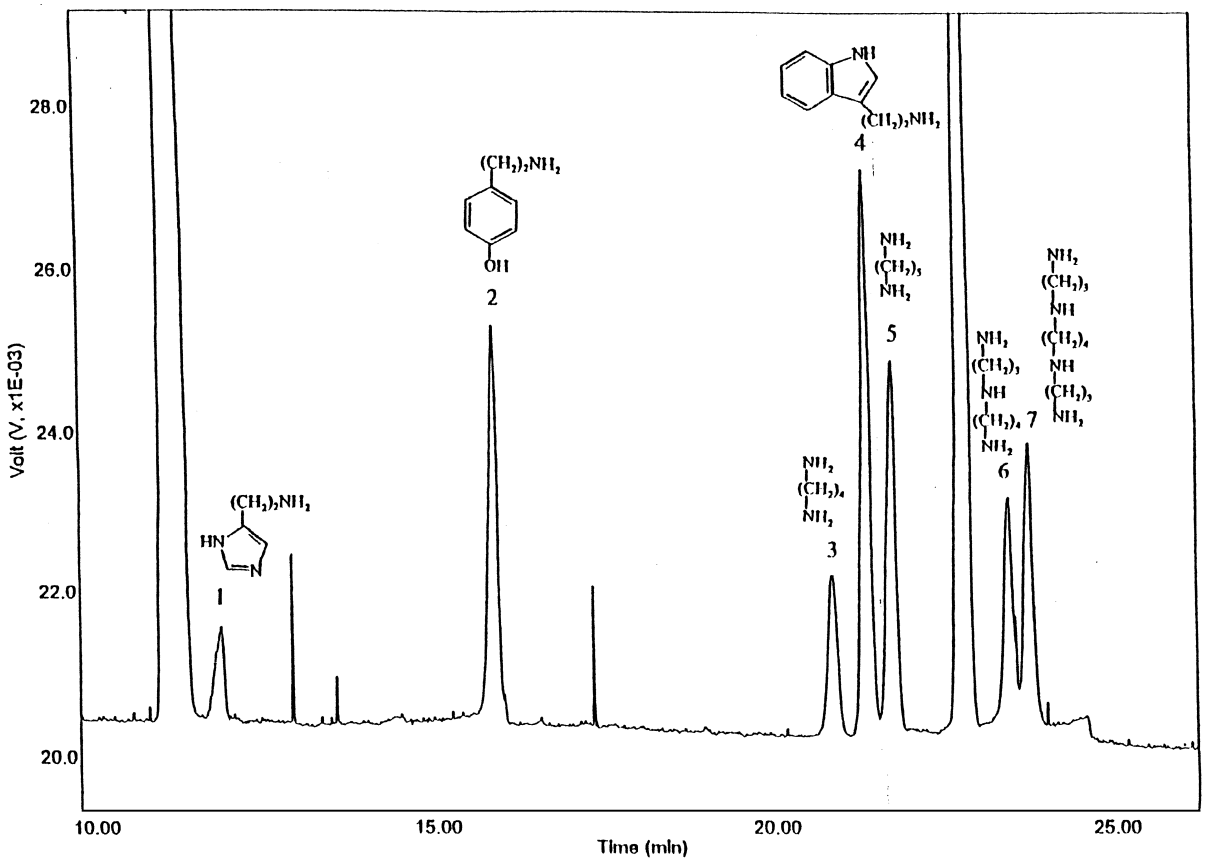

Fig. 8. Electropherogram of biogenic amine standards as 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate derivatives. Conditions 50 m*M* SDS, 10% acetonitrile, pH 8.9, 15 kV, 254 nm, fused-silica capillary 55 cm (30 cm to the detector)×50 μm I.D. at room temperature. Peaks: (1) histamine, (2) tryamine, (3) Put, (4) tryptamine, (5) Cad, (6) Spd, (7) Spm. Above the peaks the chemical structure of undelivered biogenic amines can be seen. From Ref. [224].

need for successful development of commercial instruments to open up novel methods for high-speed separations for clinical analysis.

6. Conclusion

Di- and polyamines have a multiple functions in biological systems. Monitoring the concentration of polyamines in urine, serum or affected tissue of the cancer patients at regular intervals could be of clinical significance in predicting therapeutic success or indicating relapse of tumor. For the determination of polyamines different analytical methods involving different procedures are reported. Among these methods, chromatographic procedures are commonly used. TLC of dansylated polyamines provided simple procedure for their separation and identification. Quantitation is also possible with densitometry or fluoresence scanning. Furthermore, packed column GC has been used for the effective determination of polyamines, but capillary GC showed better resolution where different fluorinated acid anhydrides are used as derivatizing reagents. On the other hand, HPLC has been more reported for polyamines determinations using post- and precolumn derivatization where postcolumn derivatization indicates somewhat broader peaks with decrease in the sensitivity due to dilution effect. An automated amino acid analyzer with fluorometric detection, following Villanueva procedures, analytical separation of polyamines is obtained with an analysis time of 110 to

160 min. HPLC with postcolumn derivatization facility with the Seilar and Knodgen method seems to give reproducible results and has been adapted by a number of laboratories. Precolumn derivatization with OPA gives better sensitivity, particularly with automated systems. Benzoyl chloride could be used when sensitivity is not demanding. In spite of limitations, dansyl chloride is the more reported reagent for precolumn derivatization. FMOC-Cl indicates necessary sensitivity, but the strongly fluorescent reagent has to be removed or masked with a suitable reagent.

6-Aminoquinoyl-*N*-hydroxysuccinimidyl and related compounds are quite promising, but the reagents have to be applied by different laboratories to evaluate them critically.

Capillary electrophoresis indicates high sensitivity for the determination of polyamines, but it would take a few years for the manufacturers to design cheaper instruments for routine use in various clinical laboratories.

7. Nomenclature

N^1-Accad	N^1-Acetylcadaverine
N^1-Acput	N^1-Acetylputrescine
N^1-Acspd	N^1-Acetylspermidine
$N^{8\prime}$-Acspd	$N^{8\prime}$-Acetylspermidine
N^1-Acspm	N^1-Acetylspermine
Adomet	Adenosyl-methionine
Adometdc	*S*-Adenosylmethionine decarboxylase
AEOC	2-(9-Anthyl)ethylchloroformate
Cad	Cadaverine
CE	Capillary electrophoresis
DAO	Diamine oxidase
N^1,N^{12}-Dacspm	N^1,N^{12}-Diacetylspermine
N^1,N^8-Dacspd	N^1,N^8-Diacetylspermidine
FMOC	9-Fluorenylmethyl chloroformate
GC	Gas chromatography
HSQC	*N*-Hydroxysuccinimidyl-6-quinolinyl carbamate
LC	Liquid chromatography
ODC	Ornithine decarboxylase
OPA	Orthophthaldehyde
PAO	Polyamine oxidase
Put	Putrescine
PEOC	(1-Pyrenyl)ethyl chloroformate
Spd	Spermidine
Spm	Spermine
TLC	Thin-layer chromatography

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