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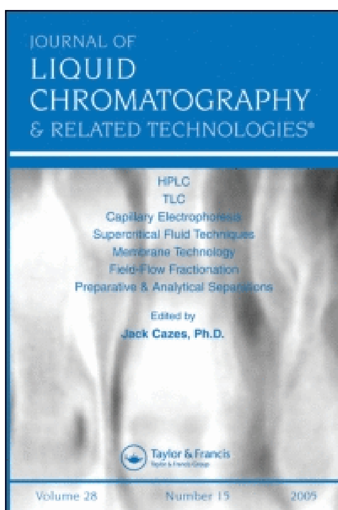

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Rapid and Simple Technique for the Quantitation of Polyamines in Biological Samples

Rodney F. Minchin[a]; Gayle R. Hanau[a]
[a] Pharmacology and Toxicology Section, Laboratory of Experimental Therapeutics and Metabolism Division of Cancer, Treatment National Cancer Institute Bethesda, Maryland

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RAPID AND SIMPLE TECHNIQUE FOR THE QUANTITATION OF POLYAMINES IN BIOLOGICAL SAMPLES

Rodney F. Minchin and Gayle R. Hanau
Pharmacology and Toxicology Section,
Laboratory of Experimental Therapeutics and Metabolism
Division of Cancer Treatment
National Cancer Institute
Bethesda, Maryland, 20205

ABSTRACT

A rapid and simple technique has been developed to quantify putrescine, spermidine, and spermine in biological tissue. The method, based upon several published procedures, involves protein precipitation with perchloric acid followed by dansylation with 5-dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride). After extraction on a Waters C_{18} Sep-Pak cartridge, the samples are analyzed by high pressure liquid chromatography using a step solvent change and a 3µ C_{18} reverse phase column. The chromatographic conditions allowed complete analysis of the three polyamines within 10 min with a total run time of 13 min (sample injection and re-equilibrium of column). Standard curves were linear up to 1 µg polyamine and the coefficient of variation for the assay ranged from 4% at 1 µg polyamine per sample to 11% at 50 ng polyamine per sample. The assay is therefore both rapid and simple. Moreover, unlike other available methods, the present technique does not require duel pumps, ion pairing agents, solvent extraction or a gradient control system. The concentrations of putrescine, spermidine and spermine in rat lung, liver and kidney are reported.

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INTRODUCTION

Polyamines are ubiquously distributed endogenous compounds that have been associated with the regulation of numerous biological functions such as DNA and RNA synthesis (1), cellular proliferation (2), differentiation (3), intracellular membrane fusion (4), protein kinase activity (5) and mitochondrial membrane activities (6). Moreover, elevated levels of polyamine have been associated with tissue injury (7,8) and certain forms of cancer in man (9), and it has been suggested that urinary or plasma polyamine levels may be a useful clinical diagnostic tool for the progression of such cancers (9).

In the present paper, a simple and rapid method for the simultaneous determination of putrescine, spermidine and spermine has been described.

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Several excellent high pressure liquid chromatographic techniques for polyamine determinations have been described (10,11). However, all available methods require elution with a solvent gradient and can take between 25 and 60 min for each assay (including re-equilibration of the column) often with poor resolution when the polyamines are extracted from biological tissue. The present technique does not require a solvent gradient system, ion pairing agents, buffers, solvent extraction or extensive reaction periods, and the chromatographic procedure is essentially complete in less than 10 min.

METHODS

Extraction of polyamines - The lungs, liver and kidneys from 250 g male Sprague Dawley rats were homogenized in 9 vol 50 mM phosphate buffer using a teflon-glass homogenizer. An aliquot (1.8 ml) of each sample was vortexed with 0.2 ml 60% perchloric acid and centrifuged at 3000 g for 4 min. The resulting supernatant (0.9 ml) was mixed with 50 μl K_2CO_3 (400 mg/ml) and 50 μl 1,6-diaminohexane (Internal standard: 500 μg/ml) and centrifuged as above. The polyamines were then dansylated by adding 40 μl of supernatant containing the internal standard to 100 μl K_2CO_3, 800 μl distilled water and 2 ml dansyl chloride (2 mg/ml in acetone). The mixture was vortexed and incubated at 60°C for 60 min in the dark. The entire sample was then placed on a C_{18} Sep-pak cartridge (Waters Assoc., U.S.A.) and washed with 9 ml 20% methanol in water. The dansylated polyamines were then eluted with 5 ml 100% methanol and 10-100 μl of this fraction was directly analyzed by HPLC.

Chromatographic conditions - An Altex 3μ-ODS (4.6 mm ID x 7.5 cm) column was equilibrated with 75% methanol in water. Following injection of sample, the solvent was changed to 95% methanol in water at 2.5 min and then back to 75% methanol at 8 min. The specific times for changing solvents were selected to minimize run time and was a function of the volume of the HPLC system. The dansylated polyamines were detected by flourescence using a Kratos FS950 detector fitted with an FSA 100 mercury lamp and FSA 403 excitation filter (Kratos Instruments, U.S.A.). A 470 mm emission filter was also used.

RESULTS AND DISCUSSION

Figure 1 illustrates a typical chromatographic profile for putrescine, 1,6-diaminohexane, spermidine and spermine (1 μg in 40 μl initial sample). The

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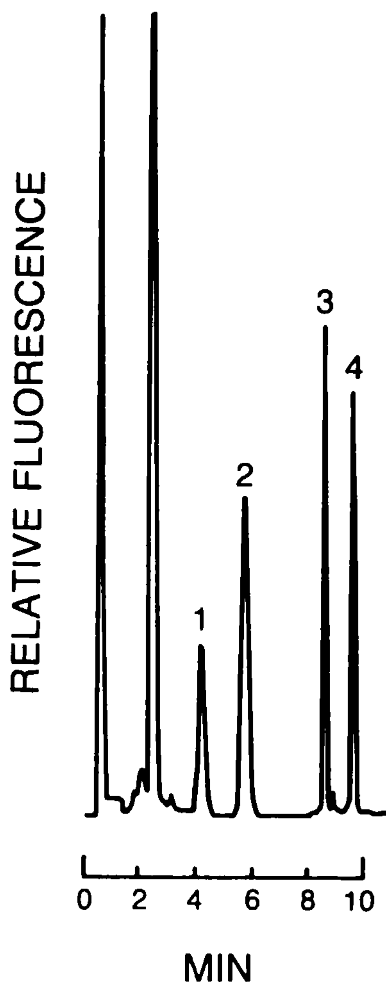

Figure 1. Chromatographic profile of polyamine standards. 1 = putrescine, 2 = 1,6-diaminohexane, 3 = spermidine, 4 = spermine.

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retention times for each polyamine were: putrescine, 3.75 min; 1,6-diaminohexane, 5.46 min; spermidine, 8.43 min; spermine, 9.34 min. Standard curves constructed over the range of 50 ng - 1 μg/sample were linear and exhibited a coefficient of variation of 4% at the upper concentrations and 11% at the lower concentrations.

The dansylation of the polyamines was not enhanced by increasing the concentration of dansyl chloride or by extending the incubation period. The yellow color of the dansyl chloride had almost completely disappeared after 60

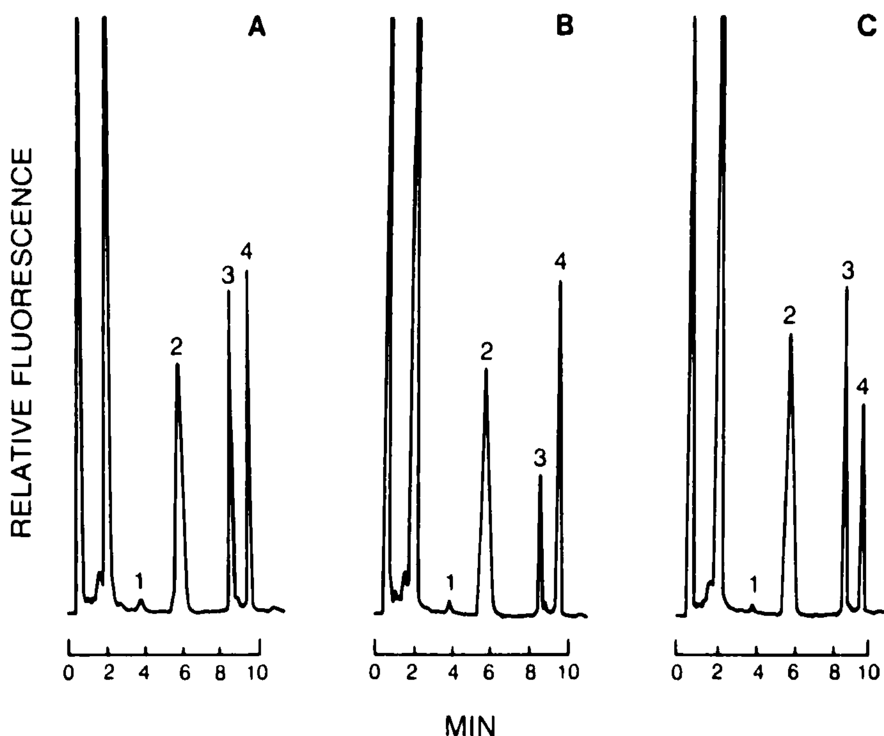

Figure 2. Chromatographic profile of polyamines extracted from rat liver (A), lung (B) and kidney (C). 1 = putrescine, 2 = 1,6-diaminohexane, 3 = spermidine, 4 = spermine.

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min. It was found that termination of the reaction before this time resulted in the elution of an excessively large peak at 1.89 min which interfered with the chromatographic profile of putrescine. The change in solvents was optimized for minimum elution time, including the time necessary for the re-equilibration of the column, without compromising the resolution between peaks. Use of a 3μ C_{18} column of only 7.5 cm in length and minimal tubing between the pump, injector, column and detector was also essential to limit the total time for each assay. Under the conditions described, a minimum of 13 min was required between each injection.

The chromatographic profiles for polyamines extracted from rat liver, lung and kidney are shown in figure 2. Putrescine levels were minimal compared to those of spermidine and spermine. The illustrated profiles could be readily

TABLE 1

Concentration of Polyamines in Rat Tissues

Tissue	Putrescine (nmol/g)	Spermidine (nmol/g)	Spermine (nmol/g)
Liver	72 ± 10*	925 ± 51	813 ± 45
Kidney	66 ± 8	506 ± 45	855 ± 91
Lung	67 ± 6	709 ± 50	458 ± 70

Results are expressed as Mean ± S.E., n=4, except* where n=3.

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enhanced by concentrating the dansylated polyamine solution under a stream of nitrogen. The estimated concentrations of putrescine, spermidine and spermine in each tissue are listed in table 1 and the reported values are within the range of published values (12).

The present study has developed a simple and reliable technique for the estimation of putrescine, spermidine, and spermine in biological tissue. The method is also rapid compared to established procedures but does not compromise peak resolution. The described technique may be useful for routine clinical determination of the polyamines for diagnostic purposes. Automation of the solvent changes can be readily achieved with an Autochrom solvent selector (Rainin Instrument, U.S.A.) and an appropriate time-dependent signal generator such as a standard HPLC data integrator.

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