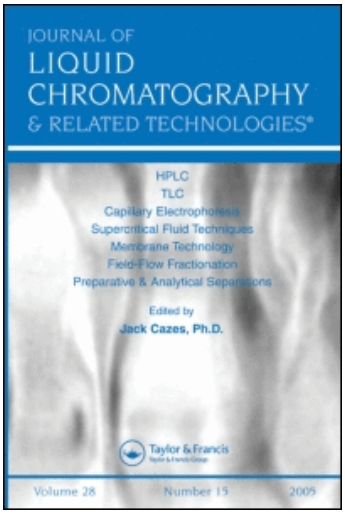

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Urinary Tyramine Assay by High-Performance Liquid Chromatography

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To cite this Article Scaro, Judy , Morrisey, Jerry L. and Shihabi, Z. K.(1980) 'Urinary Tyramine Assay by High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 3: 4, 537 — 543
To link to this Article: DOI: 10.1080/01483918008059673
URL: http://dx.doi.org/10.1080/01483918008059673

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(4), 537-543 (1980)

URINARY TYRAMINE ASSAY BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

An assay for urinary tyramine based on its reaction with fluorescamine and subsequent separation on reverse phase column has been described. The method is simple, sensitive and free from interferences. Patients with neuroblastoma, pheochromocytoma and Parkinson's disease have elevated levels of urinary tyramine.

INTRODUCTION

Tyramine is an indirect sympathomimetic amine whose physiological action is not well understood but is thought to stem from its ability to release norepinephrine from storage. Under normal conditions, trace amounts of this compound are detected in the urine. Fermented foods such as aged cheese contain high levels of tyramine and can affect the urinary level of this compound.

Tyramine has been implicated in some clinical cases such as migraine headaches (1), depression (2) and Parkinson's disease (3). Because of the clinical importance of tyramine a simple, reliable method for its assay in urine is desirable. Previous methods for its measurement include paper (4) and thin-layer (5,6) chromatography which require many steps of extraction and concentration.

In this work we describe a simple method for the assay of urinary free tyramine based on its reaction with fluorescamine and subsequent separation on a microparticulate reverse-phase column. The method is sensitive and free from interference. Furthermore, we illustrate that urines from patients with

pheochromocytoma, neuroblastoma and Parkinson's disease have elevated levels of tyramine.

MATERIALS AND METHODS

A pump, Model 110 A (Altex Scientific, Inc., Berkeley, CA 94710), was used to deliver the solvent through a 250 mm x 4.6 mm (i.d.) column of Lichrosorb RP 18, 10 um a.v. particle size (E. Merck, Cincinnati, OH 45212), at a flow rate of 1.8 ml/min. The samples were introduced through a 30 ul loop injector, Model 7120 (Rheodyne, Inc., Berkeley, CA 94710). The effluent was monitored with a Fluorescence detector (Gilson Medical Electronics, Middleton, WI 53562) using a 390 nm filter for excitation and a 475 nm filter for emission.

Procedure

A 25 ul volume of urine was buffered with 10 ul of phosphate buffer, 400 mmole/l, pH 7.8 and reacted with 100 ul of fluorescamine (250 mg/l of acetone) for 3 min at room temperature. An aliquot of 30 ul was injected onto the column and eluted with a mobile phase of 250 ml of acetonitrile diluted to a liter with phosphate buffer, pH 7.0, 13 mmole/l.

RESULTS AND DISCUSSIONS

Figure 1 illustrates chromatograms of an aqueous tyramine standard and a urine sample. The retention time for tyramine is 7 min. The method is linear between 50-3000 ug/liter (Fig. 2) and the average recovery of tyramine (1000 ug/l) added to urine was 97% $\pm$ 7.1, n=10. The minimum detectable limit is between 50-100 mg/l depending on the urine. Some urine samples contain large amounts of contaminants which elute very close to the tyramine peak. Other compounds which might interfere in the assay are listed in Table 1. Dopamine elutes slightly ahead of tyramine (Fig. 1) and elevated levels can be assayed by the same method using lower acetonitrile concentration.

The normal range for tyramine by this method based on 63 samples from adults is 0-500 ug/liter (mean 135 $\pm$ 144), 0-500 ug/g creatinine and 0-600 ug/24 h., Table 2. Since fermented foods, especially aged cheese (7), are rich in tyramine

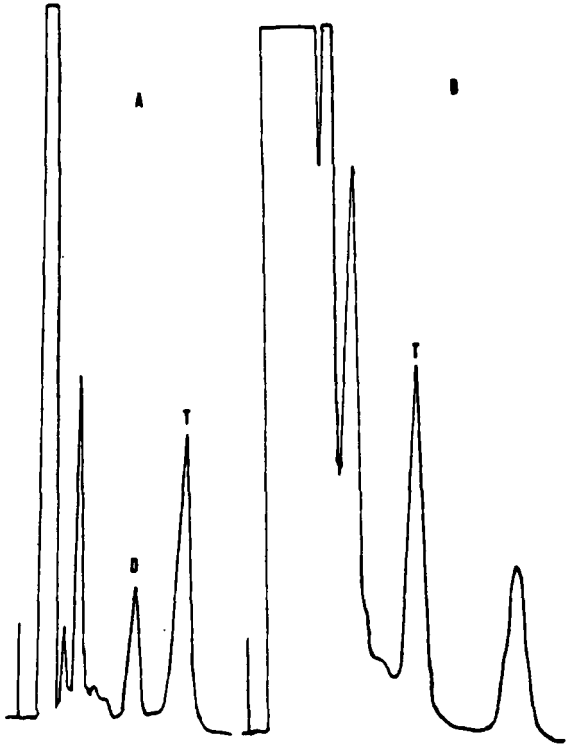

Figure 1

A - Tyramine (T) standard of 1000 µg/liter ;(D),Dopamine .

B - Tyramine (T) from the urine of a patient.

(Retention time for Tyramine is 7 min.)

and could affect the urine level of this compound, three healthy individuals collected 24 hr urine for tyramine assay before and after the consumption of 100 g of aged Cheddar cheese. Table 3 illustrates that the cheese consumption increased the urinary level of tyramine, however, all the values were within the normal range.

Pheochromocytoma which normally causes the elevation of urinary levels of catecholamine and 3-methoxy-4-hydroxymandelic acid also caused an increase in the urinary tyramine level in 2 out of 3 patients assayed (Table 2). About 50% of

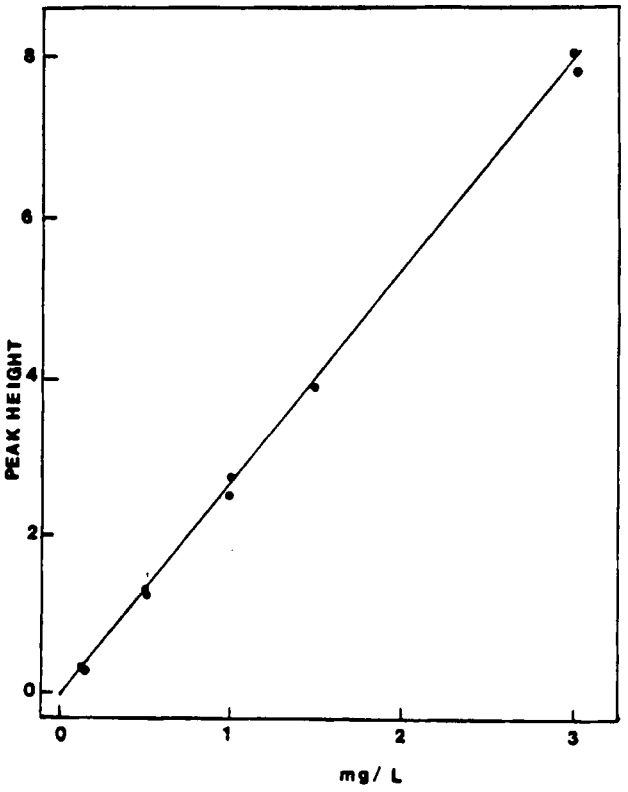

Figure 2

Tyramine linearity

the patients with neuroblastoma also had elevated levels of tyramine. Patient A with neuroblastoma had a normal level of 3-methoxy-4-hydroxymandelic acid and a borderline elevation of 4-hydroxy-3-methoxyphenylacetic acid, but his tyramine

Table 1

Retention Time for Different Compounds Related to Tyramine

Compound	Retention time in min.
Tyramine	7.0
Dopamine	5.0
Octopamine	4.4
α-methyl dopa	4.2
L-Dopa	4.3
Phenylethylamine	Does not interfere.
Tyrosine	Does not interfere.
Phenylalanine	Does not interfere.

level was about 10 fold increased. Thus the tyramine assay might be helpful in the diagnosis of the neural tissue tumors when catecholamines and their metabolites are normal.

About half of the patients with Parkinson's disease have elevated tyramine (Table 2). All these patients have elevated dopamine and 4-hydroxy-3-methoxyphenyl-acetic acid. If tyramine elevation is due to the treatment or the other factors is not clear. Smith and Kellow (3) have noticed a relationship between severity of disease in Parkinson's disease patient and the level of urinary tyramine.

TABLE 2

Urinary Tyramine Level in Normals, Patients with Pheochromocytoma, Neuroblastoma and Parkinson's Disease

Diagnosis	No. Persons	µg/liter	µg/g creatinine	µg/24 h
Normals	63	0-500	0-500	0-600
Pheochromocytoma				
D	1	5,100	920	5,340
P	1	160	120	144
S	1	2,660	9,850	6,250
Neuroblastoma				
A	1	11,200	6,200	4,816
B	1	250	2,770	360
E	1	100	1,250	710
G	1	400	1,900	820
C	1	0	0	0
D	1	0	0	0
F	1	0	0	0
Parkinson's Disease				
GL	1		6,162	
P	1		2,250	
H	1		1,612	
GI	1		1,512	
BO	1		1,304	
R	1		191	
T	1		162	
D	1		299	
E	1		402	

Table 3

The Effect of Ingestion of 100 g of Aged Cheddar Cheese on the Excretion of Tyramine (ug/g creatinine).

Individual	Before	After
SZ	120	200
MJ	150	330
MK	50	160

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