Journal of Chromatography, 570 (1991) 371–375
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5993

Short Communication

Simple and fast chromatographic method for the simultaneous determination of vanillylmandelic acid and homovanillic acid in human urine

MARIO A. ZILLI[a]

Laboratorio Analisi Cliniche, O.C. "S. Maria Della Misericordia", Udine (Italy)

(First received December 27th, 1990; revised manuscript received May 2nd, 1991)

ABSTRACT

A high-performance liquid chromatographic method for the determination of vanillylmandelic acid and homovanillic acid is described. The method is fast despite the great polarity differences between the two acids. Moreover the sample pretreatment is quick and it does not need complex or expensive equipment. The only requirement is the disposition of two pumps (or at least two eluent reservoirs) operated alternatively by means of a switching valve placed before the injection device. This makes the method available for most routine laboratories.

INTRODUCTION

Many methods have been published for the simultaneous determination of vanillylmandelic acid (VMA) and homovanillic acid (HVA) in human urine. The most appropriate technique appears to be high-performance liquid chromatography (HPLC) with electrochemical detection. Previously published methods generally require long analysis times (because of the great polarity differences between the two acids) and use time-consuming and/or expensive and complex hardware for sample pretreatment [1–9].

The method described in this paper allows a simple, quick and inexpensive sample purification, fast analyses, and requires an apparatus easily accessible to all laboratories equipped with HPLC instrumentation. Therefore it may be suited for routine determination of the two analytes.

[a] Present address: Lab. Anal. Cliniche, O.C. Monfalcone, V. Galvani 1, 34074 Monfalcone (GO), Italy.

0378-4347/91/$03.50

EXPERIMENTAL

Materials

All reagents and chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany), except for VMA, HVA and iso-VMA which were purchased from Sigma (St. Louis, MO, USA). Separation was carried out on a 12 cm × 4.6 mm I.D. stainless-steel column packed with 5 μm particle size RP-18 Sepralyte packing material (Analytichem International, Harbor, City, CA, USA).

Apparatus and chromatographic conditions

A Varian 8500 liquid chromatograph with the two pumps operated separately was used. The first (pump A) was supplied with a solution of citric acid (11.5 g/l) and the second (pump B) a mixture of the same solution containing 12% (v/v) acetonitrile. For both pumps the flow-rate was set between 1.5 and 2.0 ml/min. The access to one fixed pump was switched manually by means of a Rheodyne Model 7040 switching valve. The injector was also a Rheodyne Model 7125 supplied with a 10-μl loop.

When one of the two pumps was selected the second pump was automatically stopped without any pressure drop. This was obtained by stoppering the outlet tubing and setting the maximum pressure for that pump at a value slightly above its operating pressure.

We employed also an ESA Coulochem Model 5110 coulometric detector equipped with an analytical cell (Model 5011) under the following conditions: detector 1, +0.2 V; detector 2, +0.4 V; attenuation, 15.0 μA full scale.

After 3 min conditioning of pump A, 10 μl of the extracted samples were injected. As soon as the internal standard peak eluted, the eluent was switched from pump A to pump B, wich was operated until HVA was eluted; then, pump A was reactivated and another cycle was started. It is advisable to keep the durations of operations constant.

Sample preparation

To 1 ml of urine sample were added 100 μl of 6 *M* HCl, 40 μl of a 1 mg/ml solution of iso-VMA (internal standard) and 2.0 ml of ethyl acetate. After 2 min of vortex-mixing the mixture was centrifuged at 2122 *g*, and 1 ml of the organic phase was added to another tube containing 1 ml of a 3.4 g/l solution of potassium dihydrogenphosphate (pH *ca.* 4.55). Then samples were vortex-mixed and centrifuged again, and 10 μl of the lower phase were injected into the chromatograph.

RESULTS AND DISCUSSION

As shown in Fig. 1, only 10 min are required for elution of both analytes.

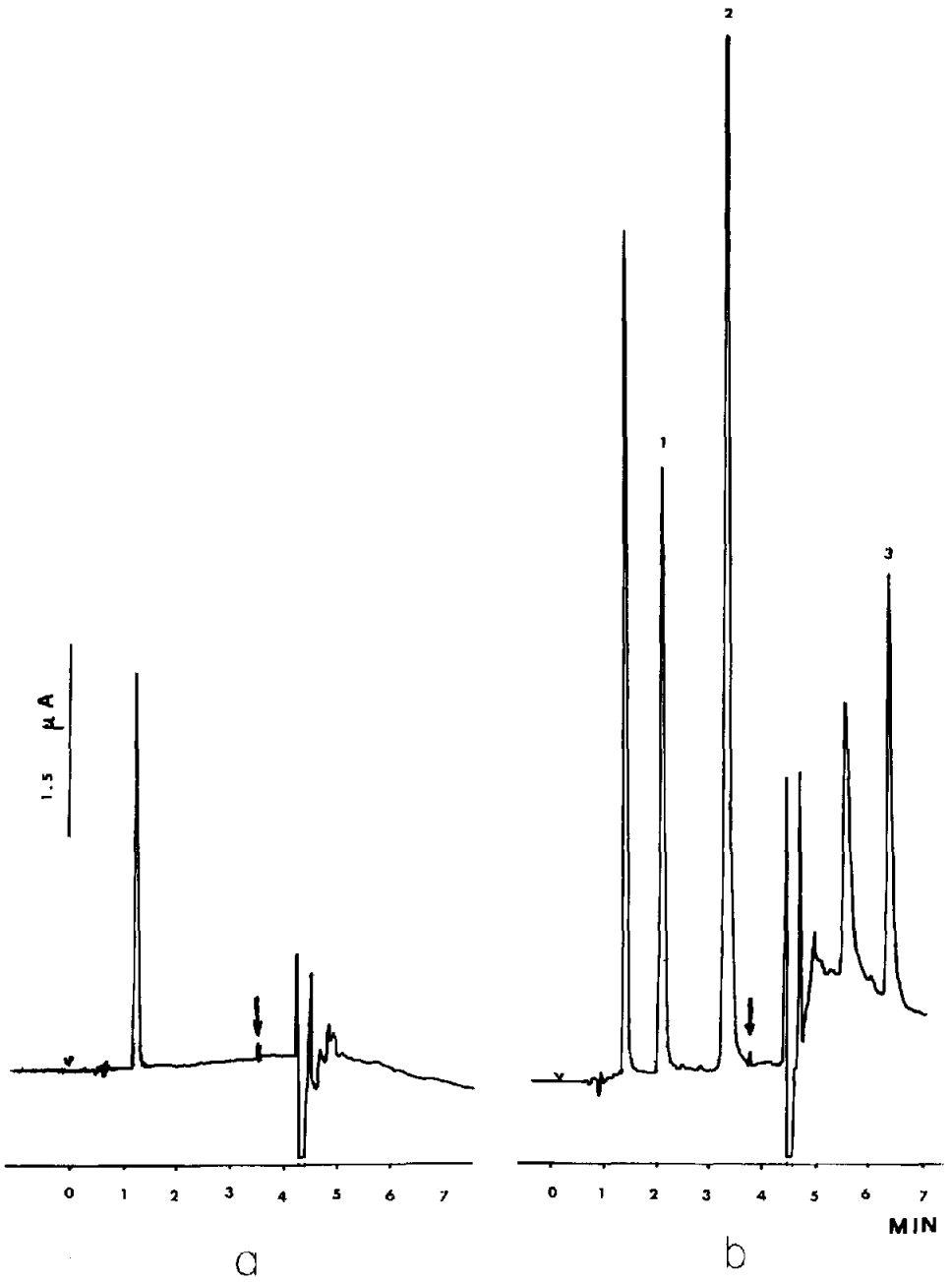

Fig. 1.

(Continued on p. 374)

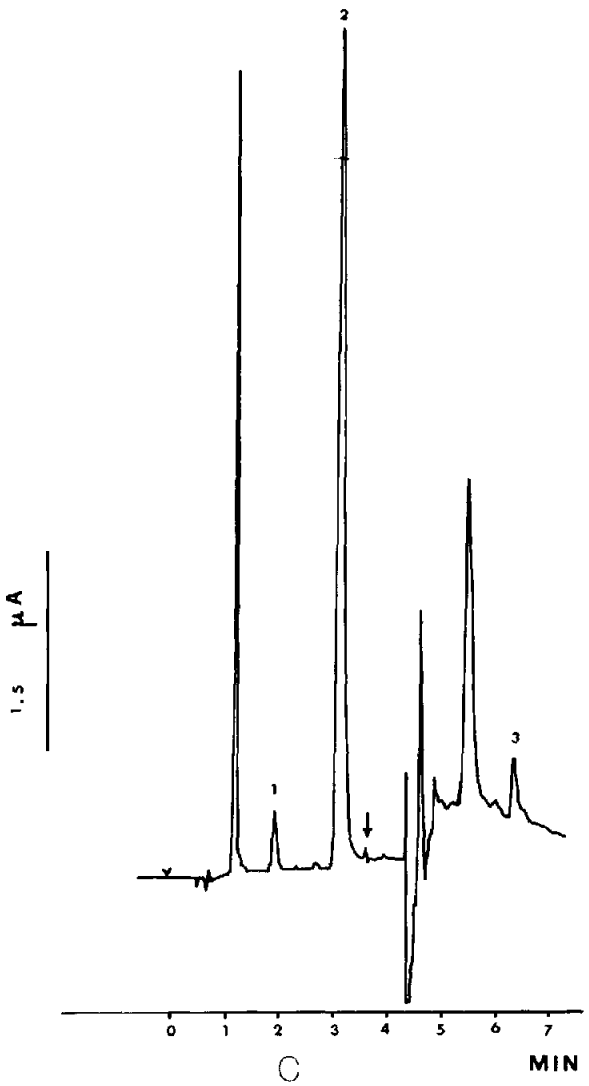

Fig. 1. Representative chromatograms of (a) a blank solution, (b) a reference solution containing 10 mg/l VMA (1), 15 mg/l HVA (3) and 40 mg/l iso-VMA (2) and (c) unspiked human urine pool. The arrows mark the change from pump A to pump B. See text for chromatographic conditions.

Moreover, when HVA is not to be determined the next sample may be injected without the use of pump B.

Quantification was performed by the standard addition method. Linearity was studied up to 20 mg/l by adding known amounts of the two acids to a human urine pool sample (r = 0.998). Within-day and day-to day precision data are shown in Table I.

TABLE I

PRECISION AND ACCURACY DATA IN WITHIN-DAY AND DAY-TO-DAY MEASUREMENTS OF THE TWO ANALYTES

Values and in mg/l; $n = 10$ in all cases.

Within-day		Day-to-day	
Added	Found (mean ± S.D.)	Added	Found (mean ± S.D.)
VMA			
0	1.05 ± 0.05	0	1.06 ± 0.07
5	6.05 ± 0.07	5	6.02 ± 0.10
10	11.1 ± 0.11	10	11.0 ± 0.21
15	15.8 ± 0.30	15	15.7 ± 0.50
HMA			
0	1.85 ± 0.11	0	1.83 ± 0.13
5	7.10 ± 0.16	5	7.02 ± 0.26
10	11.9 ± 0.18	10	11.7 ± 0.29
15	16.7 ± 0.25	15	16.6 ± 0.56

Detection limits were below 0.1 mg/l for both VMA and HVA. The extraction recovery was only *ca.* 60% for VMA and 20% for HVA, which is nevertheless sufficient to perform a precise and accurate analysis of urine samples.

Our data about the reference levels of urinary VMA and HVA agree well with those in the literature for chromatographic methods [10].

It is very important to set detector 1 at 0.2 V in order to eliminate some interfering peaks. We also found no need for complex mobile phases to achieve a good column efficiency.

The further advantages of simplicity, reliability, speed and inexpensiveness make this method of potential interest for routine assays.

REFERENCES

1 P. Wester, J. Gottfries, K. Johansson, F. Klinteback and B. Winblad, *J. Chromatogr.*, 415 (1987) 261.
2 S. Yoshikawa, S. Okuda, T. Ohe, K. Kamohara, O. Maeda and T. Sawada, *J. Chromatogr.*, 421 (1987) 111.
3 C. N. Ong, B. L. Lee, H. Y. Ong and E. Jaecob, *J. Chromatogr.*, 423 (1987) 278.
4 J. Odink, H. Korthals and J. H. Knijff, *J. Chromatogr.*, 424 (1988) 273.
5 G. R. Gotelli and P. M. Kabra, *J. Chromatogr.*, 428 (1988) 147.
6 H. Nohta, E. Yamaguchi, Y. Ohkura and H. Watanabe, *J. Chromatogr.*, 493 (1989) 15.
7 M. Koel and P. Nebinger, *J. Chromatogr.*, 495 (1989) 263.
8 Y. Kinoshita, S. Yamada, K. Haraguchi, T. Takayanagi, Y. Mori, T. Takahashi and E. Haruki, *Clin. Chem.*, 34 (1988) 2228.
9 G. A. Gerhardt, C. J. Drebing and R. Freedman, *Anal. Chem.*, 58 (1986) 2879.
10 N. W. Tietz, *Clinical Guide to Laboratory Tests*, W. B. Saunders, Philadelphia, PA, 1983.