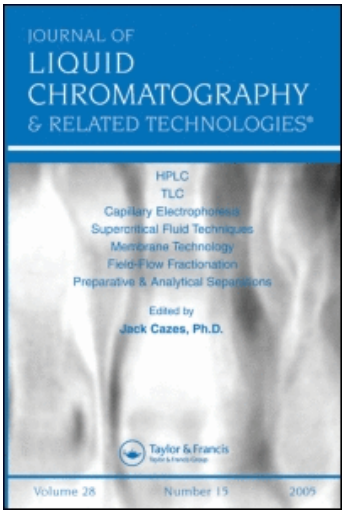

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Ren-Keui Yang M.D.[a]; James P. Edasery[b]; Kenneth L. Davis[a]

[a] Deparment of Psychiatry, Mount Sinai School of Medicine of the City University of New York, New York [b] Bronx VA Medical Center, New York

To cite this Article Yang M.D., Ren-Keui , Edasery, James P. and Davis, Kenneth L.(1983) 'A Simple Assay of 3-Methoxy-4-hydroxyphenylethyleneglycol in Cerebrospinal Fluid by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 6: 11, 1997 — 2003

To link to this Article: DOI: 10.1080/01483918308066555

URL: http://dx.doi.org/10.1080/01483918308066555

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(11), 1997-2003 (1983)

A SIMPLE ASSAY OF 3-METHOXY-4-HYDROXYPHENYLETHYLENEGLYCOL IN CEREBROSPINAL FLUID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Ren-Keui Yang, M.D.
James P. Edasery, Ph.D.
Kenneth L. Davis, M.D.

Deparment of Psychiatry,
Mount Sinai School of Medicine of the
City University of New York,
New York 10029

and

Bronx VA Medical Center
130 W Kingsbridge Road
Bronx, New York 10468

ABSTRACT

An improved method for the quantitation of 3-methoxy-4-hydroxyphenylethyleneglycol in cerebrospinal fluid is described. Sample cleaning was done by SEP PAK C_{18} Cartridge prior to the MHPG assay by high-performance liquid chromatography with electrochemical detector. The results are in good agreement wilth the GC/MS method. The average recovery is 68.3+4.1%, within run coefficient of variation of 3.8% and day to day of 7.2%. The method is simple, sensitive and accurate and can be used for routine work.

INTRODUCTION

3-Methoxy-4-hydroxythenylethylene glycol (MHPG or HMPG) is the major metabolite of norepinephrine. Its level in biological fluids and tissues has been measured in depression, anxiety and pain (1-13), obesity, hypertension and mania (14), sleep disorder (15), Gilles de la Tourette's syndrome (16) and tumors such as pheochromocytoma, neuroblostoma and ganglioneuroma (17). In plasma and urine large quantities of this metabolite exist mostly as sulfate and glucoronate conjugate. In amniotic fluid about 40% is free (18). Because of its poor volatility and thermal instability its assay involves deconjugation, extraction, addition of

 0148-3919/83/0611-1997$3.50/0

internal standard, and derivatization. Presently the assay is done by flourimetry (19), high-performance liquid chromatography (20-24), gas chromatography (25-28) and GC/mass spectrometry (29-33).

Low levels of MHPG are present in cerebrospinal fluid existing almostly completely in the free state. The compound has been assayed by direct injection into HPLC column after sample cleaning by SEP PAK C_{18} cartridge. We have simplified this HPLC assay of cerebrospinal MHPG considerably, while achieving higher sensitivity and reproductivity.

MATERIALS AND METHOD

Chemicals

3-methoxy-4-hydroxyphenylethylene glycol piperazine salt was purchased from Sigma Chemical C0. (St. Louis, MO, U.S.A.). SEP PAK C_{18} Cartridge was obtained from Waters Associates, Inc. (Milford, MA, U.S.A.). All solvents, buffer components and chemicals were of analytical reagent grade. Water was deionized and then double distilled in glass.

Apparatus

The HPLC system is made of four components. M-45 solvent delivery system (Waters Associates, Inc.); Model 7125 injection valve (Rheodyne Inc., Cotati, CA, U.S.A.). Biophase ODS 5 um C_{18} reverse phase column (Bioanalytical systems, West Lafayette, IN, U.S.A.) with an in-line guard column of 5 um RP-18 (Brownless Labs, Santa Clara, CA, U.S.A.) and LC-3 detector with a TL-5 glassy carbon electrode (Bioanalytical systems). The mobile phase consists of 0.009 mol/l citric acid and 0.089 mol/l sodium acetate buffered to pH 5.1 and contains 3% methanol. It was degassed by filteration under vacuum through a millipore 0.2 um membrane.

Procedure

The SEP PAK C_{18} Cartridge was activated by passing through 5 ml of methanol under vacuum. It was then washed with 10 ml of water to make certain

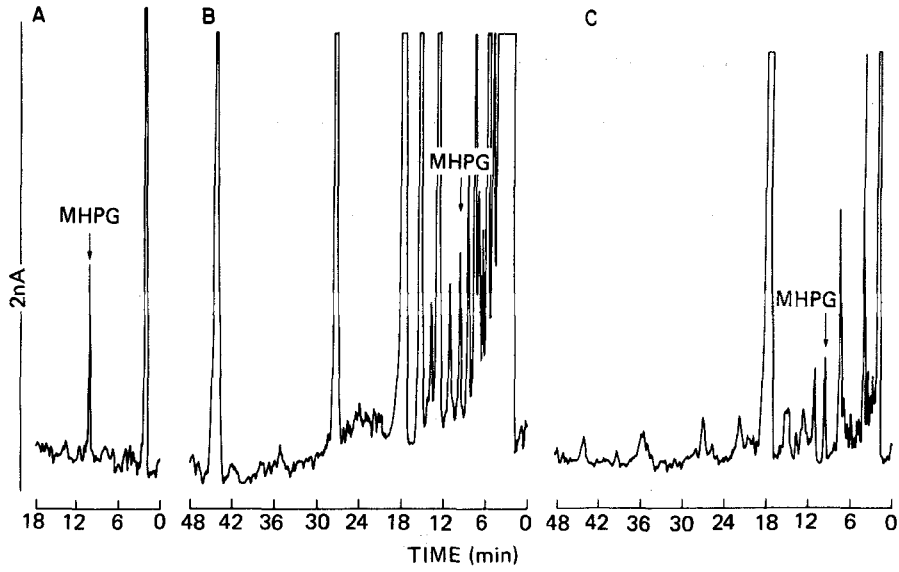

Fig. 1. Chromatograms of (A) MHPG standard (2 ng), (B) Direct injection of CSF sample, (C) CSF sample after SEP PAK cleaning. Chromatographic conditions as described under experimental.

that no methanol was left in the cartridge. The CSF sample (1 ml kept in ice) was then filtered slowly through the cartridge and washed wilth 5 ml. water. A second wash with 0.2 ml mobile phase containing 50% methanol was performed. The MHPG was then elected from the SEP PAK with 1 ml of mobile phase containing 50% methanol into a 50 ml round bottomed flask with a standard joint. The contents of the flask were then evaporated at 35°C to dryness (five minutes) on a rotary evaporator. The residue was redissolved in 0.5 ml mobile phase and aliquots were injected into the HPLC column.

Standard solutions of 5 to 20 ng MHPG piperazine salt in water were prepared and passed through SEP PAK, as were the CSF samples. The same cartridge was regenerated everytime before filtering the next sample by washing it with 5 ml of methanol and then with 10 ml of water. A standard curve was drawn with peak

Table I

MHPG in CSF (ng/ml)

Patient Number	GC/MS	HPLC
1	12.5	13.0
2	13.3	14.2
3	14.4	13.2
4	14.5	15.3
5	19.0	18.7
6	12.8	13.4
7	10.7	11.5
8	12.8	11.5
9	21.6	24.0
10	16.5	15.0
Mean	14.8	15.0
S.D.	3.3	3.8

heights against different amount of MHPG injected. The GC/MS assay was done according to B. Sjoquist et al. (30).

RESULTS AND DISCUSSION

Typical chromatograms are shown in fig. 1. The retention time for MHPG is ten minutes. The results are compared with the GC/MS assay and are shown in table 1. They are in excellent agreement. The sensitivity is 1.6 ng/ml CSF and can be improved using more than 1 ml of CSF. The recovery from SEP PAK C_{18} Cartridge is 68.3±4.1% (M±S.D., N=10) for 5 to 100 ng/ml of MHPG. The within

day coefficient of variation is 3.8 % and day to day variation is 7.2% for an average value of 13.8 ng/ml of pooled CSF samples (N=15). As shown in fig. 1

SEP Pak cleaning removes many of the unwanted materials from the samples thus reduces column contamination and achieve base line separation. Since the sample is reconstituted in the mobile phase before injection into the column, the peak shape remains the same irrespective of the volume injected. On the average ten samples can be assayed in a day. The same SEP PAK can be used for several samples. The method is very simple, sensitive, inexpensive and accurate.

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