Journal of Chromatography, 489 (1989) 359–363
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4622

Note

Specific radioactivity determination of labeled eicosanoids

UN HOI DO*, D.G. AHERN and J. ILES

Lipids Group, DuPont, NEN Products, 549 Albany Street, Boston, MA 02118 (U.S.A.)

and

M. MANISCALCO and M. TUTUNJIAN

Analytical Group, DuPont, NEN Products, 549 Albany Street, Boston, MA 02118 (U.S.A.)

(First received August 16th, 1988; revised manuscript received November 17th, 1988)

Eicosanoids with known specific radioactivities are often necessary for metabolic and pharmacological studies. However, little work has been published about accurate and reliable methods of determining specific radioactivities of these biologically active compounds. Previously, we reported enzymatic determination of specific radioactivity of polyunsaturated fatty acids [1]. However, the enzymatic method has been limited to calculating the specific activities of polyunsaturated fatty acids of the $n-3$ and $n-6$ families, precluding its use on other eicosanoids.

Attempts to determine specific activities of eicosanoids by use of liquid scintillation counting techniques with gas chromatography (GC) have been time-consuming, tedious and often unreliable due to incomplete derivatization and losses due to decomposition of these products on the column.

We developed a general method of determining specific activity that can be applied to eicosanoids without derivatization. Our investigation employed a combination of a flow-through radioactive detector and a variable-wavelength UV detector with a microbore, reversed-phase high-performance liquid chromatographic (HPLC) column to detect, separate and quantify 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TxB_2), prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), 11,9-epoxymethanoprostaglandin H_2 (U-46 619) and arachidonic acid (AA) in a reproducible and sensitive manner without derivatization of eicosanoids.

0378-4347/89/$03.50

EXPERIMENTAL

Chemicals and reagents

The following radioactive eicosanoids were obtained from DuPont, NEN Products (Boston, MA, U.S.A.): [U-^{14}C]AA, [5,6,8,9,11,12,14,15-$^{3}H_8$]AA, [5,6,8,9,12,14,15-$^{3}H_7$]PGD_2, [5,6,8,11,12,14,15-$^{3}H_7$]PGE_2, [5,6,8,9,11,12,14,15-$^{3}H_8$]$PGF_{2\alpha}$, [5,8,9,11,12,14,15-$^{3}H_7$]-6-keto-$PGF_{1\alpha}$, [5,6,8,9,11,12,14,15-$^{3}H_8$]TxB_2, and [15-^{3}H]U-46 619. Acetonitrile, water, acetic acid, potassium dihydrogenphosphate and orthophosphoric acid were HPLC grade (J.T. Baker, Phillipsburg, NJ, U.S.A.). AA was purchased from Nu-Check Prep. (Elysran, MN, U.S.A.). U-46 619 and other eicosanoid standards were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.).

Chromatographic apparatus

A Waters Model 6000 A pump, modified with a micro-flow module, delivered the mobile phase to a Waters U6K injector, equipped with a 20-μl loop. Samples were applied to a microbore Ultrasphere ODS column (250 mm$\times$2.0 mm I.D., 5 μm particle size; Waters Assoc., Milford, MA, U.S.A.). UV absorbance was monitored at 190–192 nm using a Waters Lambda-Max Model 480 variable-wavelength LC spectrophotometer equipped with a 1-μl micro flow-cell, with a 1-cm path length. Absorbance changes were recorded either on a single-pen Hewlett-Packard 3390A integrator or on a dual-pen linear chart recorder set at 10 mV full scale deflection (f.s.d.) and operated at a chart speed of 5 mm/min.

Measurement of radioactivity

Radioactivity detection was achieved by using a specially modified flow-through liquid scintillation detector. Special attention in the design and fabrication of this detector minimizes dead volume and maximizes resolution of radioactive component peaks. Column effluent from the spectrophotometer outlet is combined with DuPont Biofluor scintillation cocktail in a zero-dead-volume mixing tee.

Scintillation cocktail was delivered to the mixing tee by a piston pump (Fluid Metering, Oysterbay, NY, U.S.A.) at a flow-rate of 0.6 ml/min, providing a column effluent/scintillation cocktail ratio of 1·3. The effluent–cocktail mixture was then passed through a low-volume, flat, spiral flow-cell situated between two photomultiplier tubes. The mixture was contained within the flow-cell in 0.04-cm narrow-bore PTFE tubing. The flow-cell was fabricated with sufficient turns in the spiral to achieve a cell volume of 90 μl. The flow-cell volume and surface exposure were optimized to maximize resolution.

Signals from the photomultiplier tubes were processed and converted into useful analog signals for chart recorder display by commercially available NIM module electronics. The NIM modules contained amplification, discrimination, coincidence, ratemeter and digital display/interaction features. The 10-mV signal provided by the ratemeter module could be displayed on a dual-pen chart recorder or on a data processing integrator, such as a Hewlet-Packard 3390A integrator. A very similar flow-through liquid scintillation detector for HPLC is commer-

cially available as a FLO-ONE radioactive flow detector from Radiomatic Instruments and Chemicals (Tampa, FL, U.S.A.) as well as other vendors.

HPLC analysis

Non-radioactive eicosanoids were accurately weighed in a tared container to provide a working solution of ca. 40 μg/ml. An isocratic system was developed to separate all seven components. The column was equilibrated with solvent D: acetonitrile–0.01 *M* potassium dihydrogenphosphate (pH 3.0)–acetic acid (35:65:0.05, v/v). Elution of seven eicosanoids was carried out with solvent A: acetonitrile–0.01 *M* potassium dihydrogenphosphate (pH 3.0)–acetic acid (85:15:0.05, v/v) (see Fig. 1). [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were eluted isocratically with solvent B: acetonitrile–0.01 *M* potassium dihydrogenphosphate (pH 3.0)–acetic acid (32:68:0.1, v/v). [^{3}H]6-keto $PGF_{1\alpha}$ and [^{3}H]PGD_2 were eluted with solvent C: acetonitrile–water–2-propanol–acetic acid (35:65:0.2:0.2, v/v). [^{3}H]TxB_2 was eluted with solvent D. [^{3}H]U-46 619 was eluted with solvent E: acetonitrile–0.01 *M* potassium dihydrogenphosphate (pH 3.0) (1:1, v/v).

The analysis was carried out by use of a single pump at a flow-rate of 0.25–0.4 ml/min. Quantitation was made by use of peak area calculated using peak height times width at half peak height. Plots represent the mean of at least two assays.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of seven eicosanoids using an isocratic elution with solvent A after pre-equilibrating the column with solvent D. The elution order of [^{3}H]eicosanoids was the same as that reported in the literature [2]. The minimum detectable quantity (MDQ) of the microbore HPLC–flow-through radioactivity detection (FTRD) method was determined using [^{3}H]AA and was found to be 2100 dpm or 1.3 pg (4.3 fmol).

The correlation between the peak area and the mass of each eicosanoid (PGE_2 or $PGF_{2\alpha}$) injected was linear over 12–75 ng of authentic eicosanoid standards. The peak areas of AA, 6-keto-$PGF_{1\alpha}$, PGD_2, TxB_2 and U-46 619 were also proportional to the amount of eicosanoid injected up to 30–300 ng. The standard curves of these seven eicosanoids are not presented in this paper.

Table I shows the specific radioactivities of various [^{3}H]eicosanoids based on the calibration graphs. We also determined the specific radioactivity of [U-^{14}C]AA to be 0.85 Ci/mmol (data not shown).

The comparison of the microbore HPLC–FTRD method and other methods is shown in Table II. The results indicate that the specific radioactivity of [^{3}H]PGE_2 measured by the current method and that derived from conversion of [^{3}H]PGE_2 into [^{3}H]PGB_2 and measurement of UV absorbance at 278 nm [3] are essentially identical. The specific radioactivity of [^{3}H]AA measured by the current method agrees well with that based on the UV absorbance at 234 nm formed by the action of soybean lipoxygenase on [^{3}H]AA [1].

These results demonstrate the simple and direct measurement of specific activity by microbore HPLC–FTRD method to be reliable and reproducible. The method can be generally applied to both ^{14}C- and ^{3}H-labeled eicosanoids, requir-

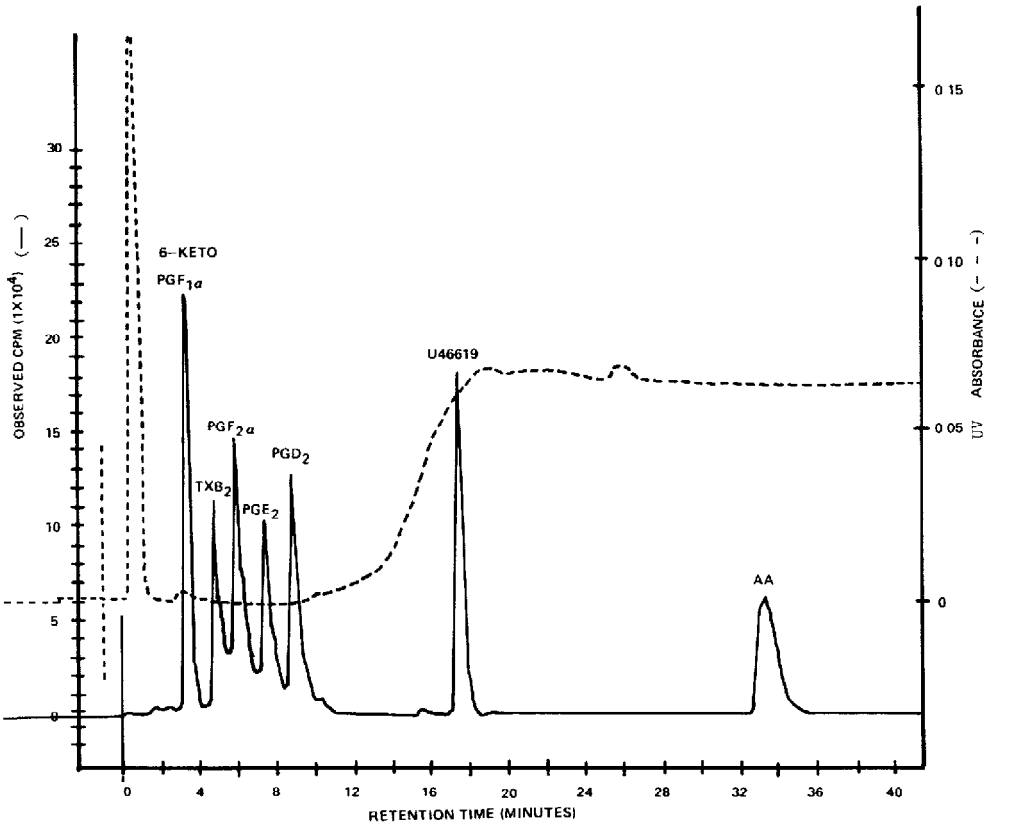

Fig. 1. HPLC of labeled eicosanoids. Column, 250 mm × 2 mm I.D. Ultrasphere ODS; linear gradient, from acetonitrile–0 01 *M* potassium dihydrogenphosphate (pH 3)–acetic acid (35:65.0.05, v/v) to acetonitrile–0.01 *M* potassium dihydrogenphosphate (pH 3)–acetic acid (85:15·0.05, v/v) in 45 min.

TABLE I

SPECIFIC RADIOACTIVITIES OF [^{3}H]EICOSANOIDS DETERMINED USING THE MICROBORE HPLC METHOD

Compound	Specific radioactivity[a] (Ci/mmol)
[5,6,8,11,12,14,15-$^3H_7$]prostaglandin E_2	206.1
[5,6,8,9,11,12,14,15-$^3H_8$]prostaglandin $F_{2\alpha}$	195.4
[5,8,9,11,12,14,15-$^3H_7$]-6-ketoprostaglandin $F_{1\alpha}$	163.5
[5,6,8,9,11,12,14,15-$^3H_8$]thromboxane B_2	213.3
[15-^{3}H]U-46 619	22.4
[5,6,8,9,11,12,14,15-$^3H_8$]arachidonic acid	191.0
[5,6,8,9,12,14,15-$^3H_7$]prostaglandin D_2	154.4

[a]The specific radioactivity was determined from triplicate samples. Agreement between triplicates was within 3%.

TABLE II

COMPARISON OF MICROBORE HPLC-FTRD METHOD WITH OTHER METHODS

Compound	Specific radioactivity[a] (Ci/mmol)	
	Microbore HPLC-FTRD method	Other methods
[5,6,8,11,12,14,15-$^3H_7$]prostaglandin E_2	206.1	210.0[b]
[5,6,8,9,11,12,14,15-$^3H_8$]arachidonic acid	191.0	186.6[c]

[a]The specific activities were determined for duplicate samples. Agreement between duplicates was within 10%.

[b][^{3}H]PGE_2 was converted into [^{3}H]PGB_2, whose specific activity was determined based on $\epsilon = 28\,650$ at 278 nm.

[c]Determined by measuring the increase in UV absorbance at 234 nm by soybean lipoxygenase reaction.

ing 1 mCi or less radioactivity. On the other hand, soybean lipoxygenase assays can be applied only to fatty acids of the $n-3$ and $n-6$ families, requiring a minimum of 60 mCi of each ^{3}H-labeled fatty acid. Likewise, few labeled prostaglandins can be converted by alkaline treatment into derivatives with strong UV absorbance, resulting in limited applications of that method to other eicosanoids.

One drawback of the method is that it is more time-consuming (average 6 h) than the UV method (average 3 h) described. The other problem with using the flow-through radioactivity detector is that the sample is lost for further characterization. However, this problem can be avoided by the use of an electronic splitter that permits the recovery and further characterization of the samples.

In summary, a combination of the microbore HPLC–FTRD was successfully employed to achieve high sensitivity and optimal separation of eight labeled eicosanoids by isocratic elution with good reliability and reproducibility. The minimum detectable quantity of the method was 1.3 pg or 4.3 fmol. The method was applicable to ^{3}H- and ^{14}C-labeled eicosanoids. The method also allows 75% lower consumption of solvent and scintillation cocktail, 80% reduction in mass requirement for mass detection and 80% reduction in amount of radioactivity required for detection compared to conventional-bore HPLC column.

ACKNOWLEDGEMENTS

We thank T. Rosenberger, P. Tam and J.R. D'Alelio for technical assistance and A. Gay for the preparation of the manuscript.

REFERENCES

1 M.A. Staples, D.G. Ahern and Un Hoi Do, Anal Biochem., 141 (1984) 316.
2 R. Rydzik, A. Terragno and R. Tackett, J. Chromatogr., 308 (1984) 31.
3 N.H. Andersen, J. Lipid Res., 10 (1969) 320.