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High-performance liquid chromatographic determination of (4-[^{11}C]methoxyphenyl)-(5-fluoro-2-hydroxyphenyl)-methyleneaminobutyric acid and its benzophenone metabolite

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Abstract

The high-performance liquid chromatographic determination of [^{11}C]MPGA {(4-[^{11}C]methoxyphenyl)-(5-fluoro-2-hydroxyphenyl)methyleneaminobutyric acid} and [^{11}C]MBENZ (5-fluoro-2-hydroxy-4′-[^{11}C]methoxybenzophenone) is described. The method was successfully applied to the quality control of [^{11}C]MPGA radiopharmaceutical preparations and for plasma analysis after i.v. injection of [^{11}C]MPGA into mice. For determination in plasma, rapid extraction with dichloromethane from buffered plasma (pH 4.5), prior to chromatography, is necessary. Separation is effected on an RP-C_{18} column, using a mixture of 0.05 *M* potassium phosphate–0.025 *M* citric acid (pH 6.5) and methanol (42:58, v/v) as the mobile phase. Detection is achieved using an ultraviolet absorption detector set at 255 nm in combination with an NaI (Tl) detector. Quantitative measurements of radioactivity are performed on a one-channel γ-ray spectrometer. The linearity, precision and accuracy for the method are also provided.

1. Introduction

γ-Aminobutyric acid (GABA) is an important neurotransmitter in the central nervous system (CNS) [1]. However, GABA is too hydrophilic to pass the blood–brain barrier readily. To solve this problem, lipophilic derivatives of GABA, e.g., progabide [**III**, (4-chlorophenyl)-(5-fluoro-2 - hydroxyphenyl)methyleneaminobutyramide] (Fig. 1), are applied [2–4]. Visualization of the GABA receptor by positron emission tomography (PET) could be an important diagnostic method in the recognition of neurological diseases. [^{11}C]MPGA {**I**, (4-[^{11}C]methoxyphenyl)-(5 - fluoro - 2 - hydroxyphenyl)methyleneaminobutyric acid} (Fig. 1) is a derivative of **III** and a potent radioligand for the study of the GABA receptor in mammalian brain by PET.

[^{11}C]MPGA (**I**) is synthesized in a two-step reaction and purified by semi-preparative HPLC. The synthesis involves O-methylation of HBENZ (**V**, 5-fluoro-2-hydroxy-4′-hydroxybenzophenone) (Fig. 1) with [^{11}C]iodomethane to form [^{11}C]MBENZ (**VI**, 5-fluoro-2-hydroxy-4′-[^{11}C]methoxybenzophenone) (Fig. 1). A subsequent Schiff reaction with γ-aminobutyric acid yields **I** [5]. After synthesis, the chemical and radiochemical purity of the **I** preparation has to be determined. Moreover, for receptor studies in the brain, the specific activity of the radiopharmaceutical preparation is also an important parameter.

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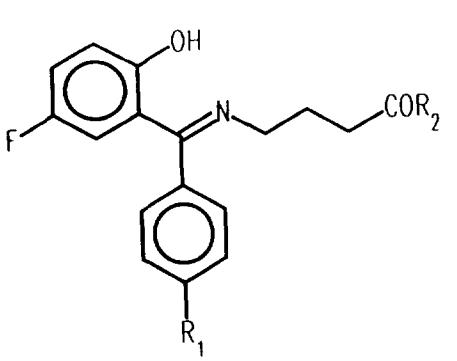

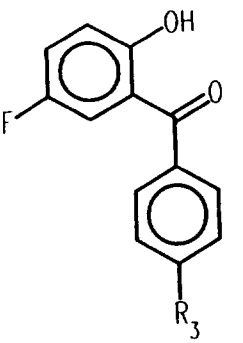

(I)	[^{11}C]MPGA	R_1=O$^{11}CH_3$	R_2=OH	(V)	HBENZ	R_3=OH
(II)	MPGA	R_1=OCH_3	R_2=OH	(VI)	[^{11}C]MBENZ	R_3=$^{11}CH_3$
(III)	progabide	R_1=Cl	R_2=NH_2	(VII)	MBENZ	R_3=OCH_3
(IV)	progabidic acid	R_1=Cl	R_2=OH			

Fig. 1. Structures of [^{11}C]MPGA (**I**), MPGA (**II**), progabide (**III**), progabidic acid (**IV**), HBENZ (**V**), [^{11}C]MBENZ (**VI**) and MBENZ (**VII**).

Once administered, radiopharmaceuticals, like any other compound, may be liable to rapid metabolization [6]. When, however, quantitative PET data regarding receptor density and affinity are needed, information on the fraction of unchanged radiopharmaceutical in the body must be available. The imine link of [^{11}C]MPGA can, analogously to progabidic acid [**IV**, (4-chlorophenyl)-(5-fluoro-2-hydroxyphenyl)methyleneaminobutyric acid] (Fig. 1), be broken down in vivo to release GABA and **VI**.

Considering the short half-life of ^{11}C ($t_{1/2}$ = 20.4 min, β-ionization), a fast and reproducible specific assay for the simultaneous determination of **I** and **VI** in radiopharmaceutical preparations and plasma is required.

Many methods have been proposed for the determination of **III** and **IV**, including GC with electron-captive detection and HPLC with UV or electrochemical detection [7–10]. These analyses were unable to detect the benzophenone metabolite. Only two methods for the simultaneous determination of progabide, progabidic acid and benzophenone have been described [11,12].

This paper describes a rapid HPLC procedure for the separation of **I** and **VI** in combination with UV and NaI(Tl) detection. The developed method was successfully applied to the quality control of radiopharmaceutical preparations and to plasma analysis for **I** and **VI** after i.v. administration to mice.

2. Experimental

2.1. Chemicals

MPGA (**II**), MBENZ (**VII**) and HBENZ (**V**) were synthesized in our laboratory and identified by ^{1}H NMR and mass spectrometry [5]. [^{11}C]MPGA (**I**) was synthesized according to a procedure described elsewhere [5]. Citric acid, methanol and dichloromethane were obtained from Janssen Chimica (Beerse, Belgium). Mono-

potassium phosphate and sodium hydroxide were obtained from UCB (Leuven, Belgium).

2.2. Equipment

The isocratic HPLC equipment consisted of a Waters Model 590 pump (Millipore, Waters Chromatography Division, Milford, MA, USA), a UV detector (Pye Unicam, Cambridge, UK), a syringe injector equipped with a 20-μl loop (Valco Instruments, Eke, Belgium), a CR5-A automatic integrator (Shimadzu, Tokyo, Japan) and an NaI(Tl) detector (Mini Instruments, Essex, UK). The eluate was collected in fractions with a Redifrac fraction collector (Pharmacia Biotech, Brussels, Belgium). Radioactivity counting was done with a one-channel γ-ray spectrometer equipped with an NaI(Tl) detector (Canberra, Meriden, CO, USA).

2.3. Chromatographic conditions

A LiChrospher 5-μm RP-C_{18} column (150 × 4.0 mm I.D.) (Merck, Darmstadt, Germany) was used. The mobile phase consisted of a mixture of 0.050 *M* monobasic potassium phosphate–0.025 *M* citric acid buffer (adjusted to pH 6.5 with sodium hydroxide) and methanol (42:58, v/v). The flow-rate was set at 1.0 ml/min. UV detection was achieved at 255 nm. The sensitivity of the UV detector was set at 0.08 AUFS. The radiochromatogram was plotted as log(cpm) versus time. Analyses were performed at ambient temperature.

For quality control of the [^{11}C]MPGA (**I**) radiopharmaceutical preparations, 20 μl were injected into the HPLC system immediately after synthesis. For the determination of the amount of carrier MPGA (**II**), the peak area was compared with a calibration graph.

A stock standard solution of **II** was prepared in methanol and stored at 2–6°C. Working standard solutions were prepared freshly by diluting the stock standard solution with mobile phase. A linear calibration graph of peak area versus concentration of **II** (2–40 μg/ml) was constructed according to the least-squares method.

2.4. Plasma analysis of [^{11}C]MPGA (I)

A 250-μl plasma sample was pipetted into a conical glass tube and 25 μl of standard solutions of **II** (20 μg/ml in methanol) and **VII** (20 μg/ml in methanol) and 250 μl extraction buffer (0.25 *M* citric acid in water adjusted to pH 4.5 with sodium hydroxide) were added. The mixture was gently mixed for 1 min and extracted with 1 ml of dichloromethane by vigorous shaking on a vortex mixer for 2 min. After centrifugation (3 min, 1000 *g*) the organic phase was separated and evaporated under a gentle stream of nitrogen. The residue was reconstituted in 50 μl of mobile phase and 20 μl were injected immediately into the HPLC system. The eluate was collected with a fraction collector at time intervals of 0.5 min. The collected fractions were counted for radioactivity on a one-channel γ-ray spectrometer.

3. Results and discussion

3.1. Chromatographic conditions

For our purposes a good separation of **II** and **VII** is necessary. Fig. 2 shows the chromatogram of a mixture of the mentioned compounds. The retention times of **II** and **VII** were 3.52 and 10.7 min, respectively ($k' = 2.63$ and 10.0, respectively; the hold-up time was determined with water). The chromatographic conditions provide a good resolution of both peaks and allow the identification and determination of **II**. Moreover, **I** and **VI** can be isolated for radioactivity counting in a γ-ray spectrometer.

As previously reported, the imine binding in GABA derivatives is unstable in aqueous media [13], undergoing hydrolysis to benzophenone derivatives and GABA. Maximum hydrolytic stability is reached at pH 7. The stability of an aqueous standard solution of **II** was controlled by injecting 20 μl every 15 min for 1 h. No change in peak height or peak area was observed during storage at 2–6°C.

A linear correlation for **II** was found between the peak area and the amount of product in-

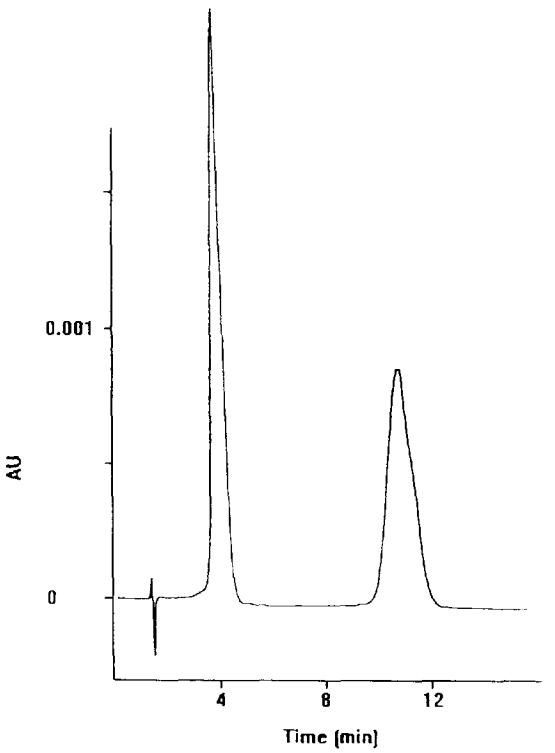

Fig. 2. Chromatogram with UV detection obtained after injection of 20 μl of a mixture containing MPGA (**II**) (20 μg/ml) and MBENZ (**VII**) (20 μg/ml).

jected in the range 2–40 μg/ml [regression equation expressed as area = slope × concentration (μg/ml) + intercept; slope = 3636, intercept = −3110, $r = 0.9996$].

The accuracy of the method was tested by an intra-assay precision test and the reproducibility by an inter-assay precision test. The choice of the concentrations used was based on the minimum and maximum levels of carrier **II** that could be found in a radiopharmaceutical preparation. The results are given in Tables 1 and 2. The recovery for **II** lies between 98.8 and 100.8% and the mean relative standard deviation is 2.01%.

It was found that 1.74 GBq (S.D. 0.218 GBq, $n = 3$) of **I** could be produced with a specific activity of 7.77 GBq/μmol (S.D. 3.19 GBq/μmol, $n = 3$). None of the products showed impurities in the UV trace or radiochromatogram. A typical radiochromatogram is shown in Fig. 3.

Table 1
Accuracy for the determination of MPGA (**II**) in radiopharmaceutical preparations ($n = 3$)

Amount added (μg/ml)	Amount found (μg/ml)	S.D. (μg/ml)	Recovery (%)
9.61	9.50	0.25	98.8
19.2	19.4	0.41	101.0
39.4	38.8	0.90	100.8
Mean			100.2

Table 2
Precision of the determination of MPGA (**II**) in radiopharmaceutical preparations ($n = 3$)

Amount added (μg/ml)	Amount found (μg/ml)	S.D. (μg/ml)	R.S.D. (%)
9.61	9.70	0.19	1.95
19.2	19.5	0.36	1.84
38.4	38.0	0.85	2.24
Mean			2.01

3.2. Plasma analyses for [^{11}C]MPGA (I)

The extraction of [^{11}C]MPGA (**I**) from plasma obtained from mice injected with 3.7 MBq, corresponding to approximately 130 ng of car-

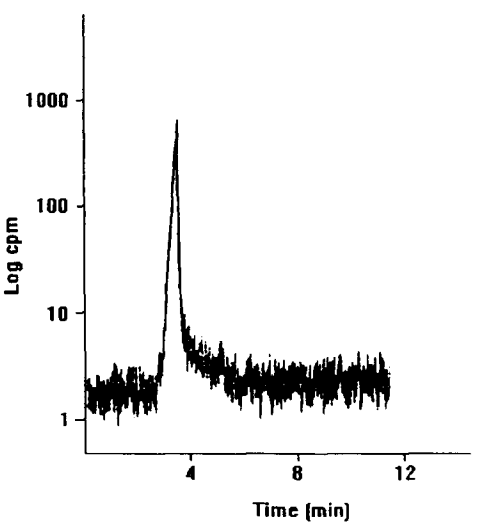

Fig. 3. Typical radiochromatogram obtained after injection of 20 μl of a [^{11}C]MPGA preparation.

rier, was not reproducible. Therefore, 500 ng of both standard **II** and **VII** were added to the plasma before extraction. The recovery was determined by comparing the peak areas of **II** and **VII** in the chromatogram with the peak areas of the respective standard solutions. Recoveries of **II** and **VII** of 90 and 91%, respectively, were obtained.

The stability of **I** during the extraction was controlled by HPLC. The radiochromatogram of a blank plasma spiked with 37 kBq of **I** and treated as described under Experimental showed only one peak with the same retention time as **II**, indicating that no hydrolysis at the imine binding took place during the extraction procedure.

The radiochromatogram of a typical mouse plasma sample extract is shown in Fig. 4. All radioactivity corresponding to **I** is present in the fractions collected between 2.5 and 4.5 min and is well separated from **VI**. The fraction of unchanged **I** in plasma samples was determined by measuring the ratio of the radioactivity corresponding to **I** to the total radioactivity present in all collected fractions.

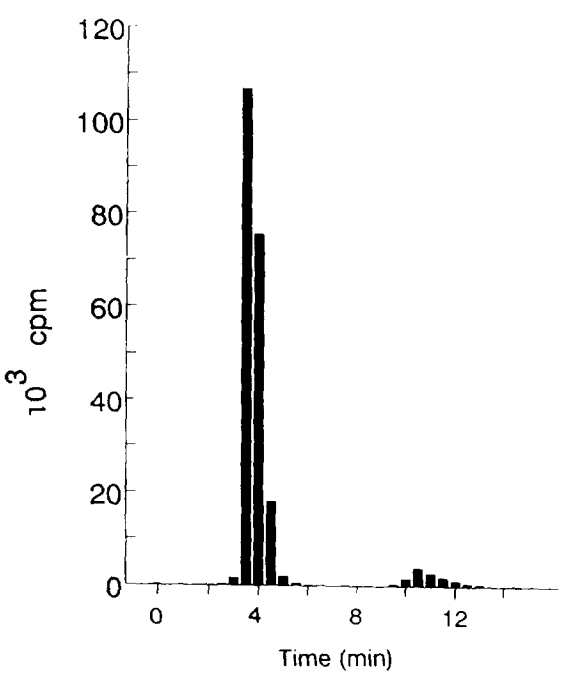

Fig. 4. Distribution of the radioactivity versus time for a mice plasma sample 30 min after administration of [^{11}C]MPGA (**I**).

A 3.7-MBq amount of **I** was administered i.v. to white male mice (strain NMRI) and blood samples were taken at 5, 10 and 30 min post-injection. Plasma was obtained by centrifugation of the collected blood at 3000 *g* for 5 min. The plasma samples were treated as described under Experimental. The fraction of unchanged **I** was 95, 97 and 94% at 5, 10 and 30 min after injection, respectively. These values indicate that up to 30 min after administration no metabolization of importance occurred. No blood samples were taken at longer times post-injection because the amount of radioactivity present in the collected HPLC fractions was too low to perform adequate radioactivity counting.

4. Conclusions

An HPLC method for the separation of MPGA (**II**) and MBENZ (**VII**) has been developed. The method was successfully applied to the quality control of [^{11}C]MPGA (**I**) radiopharmaceutical preparations and for plasma analyses for **I** and [^{11}C]MBENZ (**VI**) after i.v. administration of **I** to mice.

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