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CA 2092979 C 2004/01/27

(11)(21) 2 092 979

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 1993/03/30

(41) Mise à la disp. pub./Open to Public Insp.: 1994/05/28

(45) Date de délivrance/Issue Date: 2004/01/27

(30) Priorité/Priority: 1992/11/27 (339495/1992) JP

(51) Cl.Int.^{5}/Int.Cl.^{5} C08B 37/16, A61K 31/715

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(54) Titre : COMPLEXE DE PACLITAXEL CONTENANT DE LA CYCLODEXTRINE, METHODE DE PRODUCTION ET D'UTILISATION DE CE DERNIER

(54) Title: CYCLODEXTRIN INCLUSION COMPLEX OF PACLITAXEL, AND METHOD FOR ITS PRODUCTION AND ITS USE

(57) Abrégé/Abstract:
There is provided a cyclodextrin or branched cyclodextrin inclusion complex of paclitaxel, a method for the production of cyclodextrin of branched cyclodextrin inclusion complex of paclitaxel characterized by adding cyclodextrin or branched cyclodextrin thereto at a molar ratio of 1-20 times with respect to paclitaxel, and a method for the improvement of the solubility of paclitaxel in water by adding cyclodextrin or branched cyclodextrin thereto at a molar ratio of 1-20 times with respect to paclitaxel. According to the present invention, the solubility of paclitaxel in water may be improved, and paclitaxel may be solubilized, by adding CD thereto at a molar ratio of 1-20 times with respect to paclitaxel. If a CD inclusion complex of paclitaxel according to the present invention is used, paclitaxel administered to cancer patients may be easily absorbed, and the physiological effects of paclitaxel may be more effectively induced.

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ASTRACT

There is provided a cyclodextrin or branched cyclodextrin inclusion complex of paclitaxel, a method for the production of cyclodextrin of branched cyclodextrin inclusion complex of paclitaxel characterized by adding cyclodextrin or branched cyclodextrin thereto at a molar ratio of 1-20 times with respect to paclitaxel, and a method for the improvement of the solubility of paclitaxel in water by adding cyclodextrin or branched cyclodextrin thereto at a molar ratio of 1-20 times with respect to paclitaxel. According to the present invention, the solubility of paclitaxel in water may be improved, and paclitaxel may be solubilized, by adding CD thereto at a molar ratio of 1-20 times with respect to paclitaxel. If a CD inclusion complex of paclitaxel according to the present invention is used, paclitaxel administered to cancer patients may be easily absorbed, and the physiological effects of paclitaxel may be more effectively induced.

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CYCLODEXTRIN INCLUSION COMPLEX OF PACLITAXEL, AND METHOD FOR ITS PRODUCTION AND ITS USE

Field of the Invention

The present invention relates to a method for improving the solubility of paclitaxel in water, and more particularly, to a method for improving its water solubility by adding cyclodextrin or branched cyclodextrin (both sometimes referred to hereinafter as "CD") at a molar ratio of 1-20 times with respect to paclitaxel to obtain a CD inclusion complex of paclitaxel, thus improving the solubility of paclitaxel in water and solubilizing paclitaxel.

Description of the Prior Art

Paclitaxel is a substance which is extracted from the bark of a species of the North American yew tree (Taxus brevifolia), and it inhibits the division of cancer cells. Particularly, it is known to be effective on ovarian cancer patients, and is attracting attention as a novel and powerful anti-cancer agent. However, paclitaxel does not dissolve in water, and thus is not absorbed when administered to patients.

Paclitaxel was only recently discovered, and no reports exist as yet regarding its solubility.

Summary of the Invention

The purpose of the present invention is to overcome the above mentioned disadvantage of paclitaxel.

As a result a varied and repeated research with this in mind, we the inventors of the present invention discovered that paclitaxel can be solubilized by adding CD thereto at a molar ratio of 1-20 times with respect to paclitaxel, to

improve the solubility of paclitaxel in water. The present invention was completed on the basis of this discovery.

Brief Description of the Drawings

Fig. 1 shows an HPLC chromatogram of paclitaxel.

Fig. 2 shows an absorption spectrum of paclitaxel.

Fig. 3 shows an HPLC chromatogram of paclitaxel---CD in a case where ethanol was used as the solvent according to Example 2.

Fig. 4 shows an absorption spectrum of paclitaxel---CD in a case where ethanol was used as the solvent according to Example 2.

Fig. 5 shows a three-dimensional HPLC chromatogram of paclitaxel-maltosyl-β-CD in a case where ethanol was used as the solvent according to Example 2.

Fig.6 shows an HPLC chromatogram of paclitaxel-γ-CD in a case where ethyl acetate was used as the solvent and manual reciprocal shaking was effected according to Example 2.

Fig. 7 shows an absorption spectrum of paclitaxel-γ-CD in a case where ethyl acetate was used as the solvent and manual reciprocal shaking was effected according to Example 2.

Fig. 8 shows an HPLC chromatogram of paclitaxel-γ-CD in a case where ethyl acetate was used as the solvent and stirring was effected for one hour according to Example 3.

Fig. 9 shows an absorption spectrum of paclitaxel-γ-CD in a case where ethyl acetate was used as the solvent and stirring was effected for one hour according to Example 3.

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Detailed Description of the Invention

The present invention provides a cyclodextrin or branched cyclodextrin inclusion complex of paclitaxel, and further provides a method for the production of a cyclodextrin or branched cyclodextrin inclusion complex of paclitaxel characterized by adding cyclodextrin or branch cyclodextrin to paclitaxel at a molar ratio of 1-20 times with respect to paclitaxel, as well as a method for the improvement of solubility of paclitaxel in water by adding cyclodextrin or branched cyclodextrin thereto at a molar ratio of 1-20 times with respect to paclitaxel.

In order to obtain a CD inclusion complex of paclitaxel, paclitaxel is first dissolved in an organic solvent. Here, the organic solvent may be ethyl acetate, methanol, ethanol, acetone, etc., but ethanol is particularly preferable. Here, the amount of the organic solvent is not particularly limited so far as it dissolves paclitaxel. The organic solvent is preferably miscible with water at least to some extent.

On the other hand, an aqueous solution of CD is prepared in an amount of 1-20 moles per mole of paclitaxel, and is added to the paclitaxel solution. In this point, stirring is generally preferred for complete mixing of the solution, and more preferably, a stirrer or the like is used for vigorous stirring. Also, there are no special conditions regarding the reaction temperature, and the reaction may proceed adequately at room temperature. The reaction time may be from a few minutes to a few hours, and is normally from about 2 minutes to an hour.

Paclitaxel is clathrated (or included) in the CD by this reaction. The CD used according to the present invention

may be, for example, α-CD, β-CD, γ-CD, glucosyl-α-CD, glucosyl-β-CD, glucosyl-γ-CD, maltosyl-α-CD, maltosyl-β-CD, maltosyl-γ-CD, maltotriosyl-α-CD, maltotriosyl-β-CD, maltotriosyl-γ-CD, etc., and these may be used alone or in appropriate admixture.

If the amount of CD added is less than the lower limit of 1:1 with respect to paclitaxel, the solubility of paclitaxel is not adequately increased, and if it is greater than 1:20, the excessive amount of CD does not contribute to inclusion complex formation.

In addition, if the solution is dried, a stable CD inclusion complex of paclitaxel may be obtained, which is highly soluble in water.

When a CD inclusion complex of paclitaxel obtained in this manner is administered to a patient intravenously, orally or via another route, a minute but highly efficient dosage of paclitaxel may be provided to the patient. Thus, the physiological activity of paclitaxel may be efficiently induced.

A CD inclusion complex of paclitaxel obtained according to the present invention may be used in a variety of different pharmaceutical composition forms containing an anti-cancer effective amount of the CD complex of paclitaxel and a pharmaceutically acceptable carrier. Such pharmaceutical composition forms are generally well known in the art. For example, it may be prepared as an injection or used as a powder without further processing, or the powder may be granulated, tableted of filled into capsules, etc., in a form as to best accommodate the patient. Of course, it may also be used in the form of the original solution. Further, the same effects may

be expected even if paclitaxel is clathrated using a chemically modified CD such as hydroxypropyl CD.

The CD inclusion complex of paclitaxel obtained according to the present invention may also be used in a commercial package, comprising: (a) a pharmaceutical dosage form comprising the CD inclusion complex of paclitaxel obtained according to the present invention and a pharmaceutically acceptable carrier; and (b) a written matter describing instructions for the use thereof for treating a cancer.

Examples

A more detailed explanation of the present invention will now be given with reference to the Examples, but the present invention is not limited to these Examples.

Example 1

In this example, the influence of each type of CD on the solubilization of paclitaxel was investigated.

First, 1 mg of paclitaxel was dissolved in 2.0 ml of ethyl acetate. Meanwhile, each type of CD was dissolved in 2.0 ml of water to a molar ratio (paclitaxel:CD) of 1:1, 1:5, 1:10 and 1:20, respectively. The CDs used were α-, γ-, maltosyl-α-, maltosyl-β- and maltosyl-γ-CD.

The paclitaxel solution and the CD solutions were placed in test tubes each equipped with the ground-in stopper, and were then mixed by manual reciprocal shaking repeated 30 times. Next, the ethyl acetate layer was collected, and the absorbance was measured at 255 nm to calculate the amount of paclitaxel which moved to the aqueous layer. The solubility of

paclitaxel was calculated by subtracting the solubility thereof when CD was not dissolved in the aqueous layer (blank value), and the results are shown in Table 1. That is, since ethyl acetate used here moves to the aqueous phase and thus a minute amount of paclitaxel dissolves therein even in a blank case where no CD is used, a solubility of 0.589 mg per 2 ml of water is obtained, and therefore this value has been subtracted from the solubilities listed in Table 1. The numbers in the table,

then, represent the solubility of paclitaxel increased by the addition of CD.

Table 1

Increased solubility of paclitaxel in water using various CDs

Molar ratio (paclitaxel:CD)	1:1	1:5	1:10	1:20
α-CD	0.067	0.036	0.213	0.186
γ-CD	0.045	0.050	0.315	0.319
Maltosyl-α-CD	0.048	0.102	0.106	0.208
Maltosyl-β-CD	0.036	0.017	0.120	0.132
Maltosyl-γ-CD	0.055	0.044	0.183	0.118

(mg per 2 ml of water)

The above results show that the solubility of paclitaxel is improved by addition of CD at a molar ratio thereto of 1:1 or greater. Also, with the exception of maltosyl-α-CD, an adequate effect was obtained with addition of CD at a molar ratio of 1:10 or greater.

Further, of the various types of CD, -γ-CD in particular produced significant improvement in the solubility of paclitaxel, identifying it as the most appropriate substance for solubilization of paclitaxel.

Example 2

In this example, the solubility of CD inclusion complex of paclitaxel was determined in a more precise manner than in Example 1, and the influence of the organic solvent used to dissolve paclitaxel was also investigated.

One milligram of paclitaxel was dissolved in 2.0 ml of ethanol, and each type of CD was dissolved in 2.0 ml of water to a molar ratio (paclitaxel:CD) of 1:1, 1:5, 1:10 and

1:20, respectively. The CDs used were α-, γ-, maltosyl-α', maltosyl-β- and malotsyl-γ-CD.

The paclitaxel solution and the CD solution were placed in a test tube equipped with a ground-in stopper, and mixed by manual reciprocal shaking repeated 30 times, in the same manner as in example 1. The entire solution was frozen immediately thereafter, and then subjected to lyophilization, which completely removed the water and the organic solvent.

Next, 2.0 ml of water was added to each of the lyophilized products obtained in this manner, the mixture was stirred and centrifuged, and then the solubility of paclitaxel in the supernatant was measured by high performance liquid chromatography (HPLC). The results thereof are shown in Table 2, along with the results of a case which was effected in the same manner, but using 2.0 ml of ethyl acetate as the organic solvent in place of 2.0 ml of ethanol.

The conditions for HPLC were as follows.

Column:	Crestpack* (Jasco)
Solvent:	Methanol:water = 60:40
Flow rate:	1.0 ml/min
Column temperature:	40°C
Detector:	Ultraviolet detector

*Trade-mark

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Table 2

Influence of solvent on solubility of paclitaxel in water

	Ethyl acetate	Ethanol
α-CD	0.4	14.5
γ-CD	10.8	117.9
Maltosyl-α-CD	3.1	11.1
Maltosyl-β-CD	4.4	42.8
Maltosyl-γ-CD	8.1	38.0

(μg per mg of water)

For the paclitaxel assay, paclitaxel itself untreated with CD was dissolved in ethyl acetate, and paclitaxel solutions of various concentrations were prepared and their HPLC chromatograms were prepared. One example thereof is shown in Fig, 1, and the absorption spectrum is shown in Fig. 2. On the other hand, the HPLC chromatogram of a case where ethanol and γ-CD were used is shown in Fig. 3, and the absorption spectrums at each peak measured at the same times as indicated in Fig. 3 are shown in Fig. 4. The peak picking information for Figs. 1 and 3 are given below.

	Time (min)	AUFS	Peak wavelength
Fig. 1 (1):	17.27	0.078	230
Fig. 3 (1):	28.95	0.038	218
Fig. 3 (2):	17.27	0.015	230
Fig. 3 (3):	11.26	0.005	284
Fig. 3 (4):	3.86	0.036	214

In addition, a three-dimensional HPLC chromatogram of a case where ethanol and maltosyl-β-CD were used is shown in Fig. 5, and an HPLC chromatogram of the case where ethyl acetate and γ-CD were used is shown in Fig. 6. The absorption

spectrum measured at each peak at the times indicated in Fig. 6 are shown in Fig. 7. The peak picking information for Fig. 6 is given below.

	Time (min)	AUFS	Peak wavelength
Fig. 6 (1):	3.86	0.008	214, 238
Fig. 6 (2):	17.02	0.004	228
Fig. 6 (3):	28.78	0.006	218

As is clear from the Figures, paclitaxel to which CD was added had a different retention time and a different absorption spectrum in HPLC, compared to paclitaxel by itself. This fact indicates inclusion of the functional groups of paclitaxel by CD. It is thought that CD forms inclusion complex with the hydrophobic group of paclitaxel, thus improving the solubility thereof. There was a greater improvement in the solubility when ethanol was used as the solvent for paclitaxel, than when ethyl acetate was used. This is thought to be due to the changing of the conformation of paclitaxel by the solvent. Even when ethanol was used as the solvent instead of ethyl acetate, γ-CD significantly improved the solubility of paclitaxel, and was the most excellent solubilizer thereof.

Example 3

Twenty milligrams of paclitaxel was dissolved in 40 ml of ethyl acetate, 200 mg of γ-CD was dissolved in 40 ml of water, and the two solutions were mixed and stirred for 1 hour with the stirrer. After allowing the mixture to stand, the aqueous layer was recovered and lyophilized. To the obtained dried product was added 40 ml of water, and the mixture was stirred for dissolution and subjected to centrifugation, after which the amount of paclitaxel contained in the supernatant was

measured using HPLC. The conditions for HPLC were the same as those in Example 2. The results are shown in Fig. 8, and the absorption spectrums measured at the respective peaks at the same times as indicated in Fig. 8 are shown in Fig.9. The peak picking information for Fig. 8 is given below.

	Time (min)	AUFS
Fig. 8 (1):	16.45	0.041
Fig. 8 (2):	17.19	0.046

The results indicated an amount of paclitaxel of 81.5 μg per 1 mg of water. Comparing this to the result of 10.8 μg in Example 2 where manual reciprocal shaking was repeated 30 times, there was clearly a drastic increase in solubility. The reason for this is thought to be that the stirring time (reaction time) was 1 hour, or longer than in Example 2.

Further, when the HPLC chromatograms are compared, elution was effected at retention times 3.86, 17.02 and 28.78 minutes (Fig.6) in the case where manual reciprocal shaking was effected, whereas elution was effected at 16.45 and 17.19 minutes (Fig.8) in the case where stirring was effected for one hour. Further, the absorption spectrums converged at around 230 nm (Fig. 9). This was thought to be due to the fact that a clathration equilibrium was reached with a certain proportion of CD forming inclusion complexes of the functional groups of paclitaxel.

Thus, with a reaction time of one hour, a CD inclusion equilibrium may be reached, the solubility of paclitaxel may be improved, and the solubilization of paclitaxel may be generally increased.

As mentioned above, according to the present invention, the solubility of paclitaxel in water may be

improved, and paclitaxel may be solubilized, by adding CD thereto at a molar ratio of 1-20 times with respect to paclitaxel. Therefore, if a CD inclusion complex of paclitaxel according to the present invention is used, paclitaxel administered to cancer patients may be easily absorbed, and the physiological effects of paclitaxel may be more effectively induced.

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CLAIMS:

1. A cyclodextrin or branched cyclodextrin inclusion complex of paclitaxel, which comprises 1 to 20 moles of the cyclodextrin or branched cyclodextrin per mole of paclitaxel.

2. The inclusion complex according to claim 1, wherein the cyclodextrin is a non-branched cyclodextrin selected from the group consisting of α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and a mixture thereof.

3. The inclusion complex according to claim 1, wherein the cyclodextrin is a branched cyclodextrin selected from the group consisting of glucosyl-α-cylodextrin, glucosyl-β-cyclodextrin, glucosyl-γ-cyclodextrin, maltosyl-α-cyclodextrin, maltosyl-β-cyclodextrin, maltosyl-γ-cyclodextrin and a mixture thereof.

4. The inclusion complex according to claim 1, wherein the cyclodextrin is hydroxypropyl cyclodextrin.

5. The inclusion complex according to claim 1, wherein the cyclodextrin is γ-cyclodextrin, glucosyl-γ-cyclodextrin or maltosyl-γ-cyclodextrin.

6. A method for producing the inclusion complex as defined in any one of claims 1 to 5, which comprises:

adding an aqueous solution of cyclodextrin or branched cyclodextrin to an organic solvent solution of paclitaxel, wherein the cyclodextrin or the branched cyclodextrin is used in an amount of 1 to 20 moles per mole of paclitaxel.

7. The method according to claim 6, wherein the organic solvent in which paclitaxel is dissolved is ethyl acetate, methanol, ethanol or acetone.

8. The method according to claim 7, wherein the organic solvent is ethanol.

9. The method according to claim 6, 7 or 8, which further comprises drying off water and the organic solvent to produce the inclusion complex in a dry form.

10. A pharmaceutical composition for treating a cancer, which comprises an anti-cancer effective amount of the inclusion complex as defined in any one of claims 1 to 5, in admixture with a pharmaceutically acceptable carrier.

11. A method for the improvement of the solubility of paclitaxel in water, which comprises adding a cyclodextrin or branched cyclodextrin to paclitaxel at a molar ratio of 1-20 times with respect to paclitaxel.

12. A commercial package, which comprises:

(a) a pharmaceutical dosage form comprising the inclusion complex as defined in any one of claims 1 to 5 and a pharmaceutically acceptable carrier; and

(b) a written matter describing instructions for the use thereof for treating a cancer.

SMART & BIGGAR

OTTAWA, CANADA

PATENT AGENTS

FIG. 1

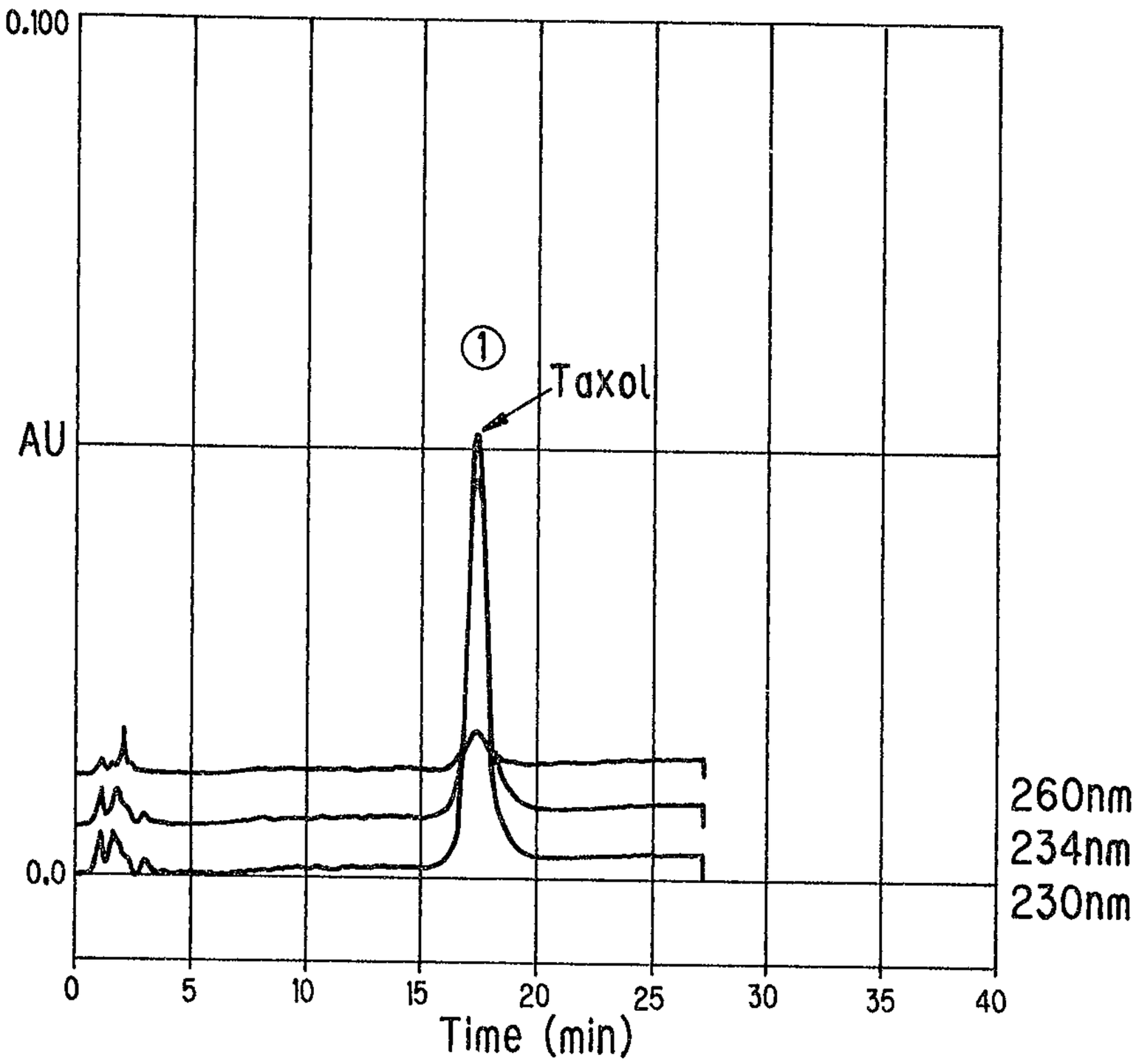

FIG. 2

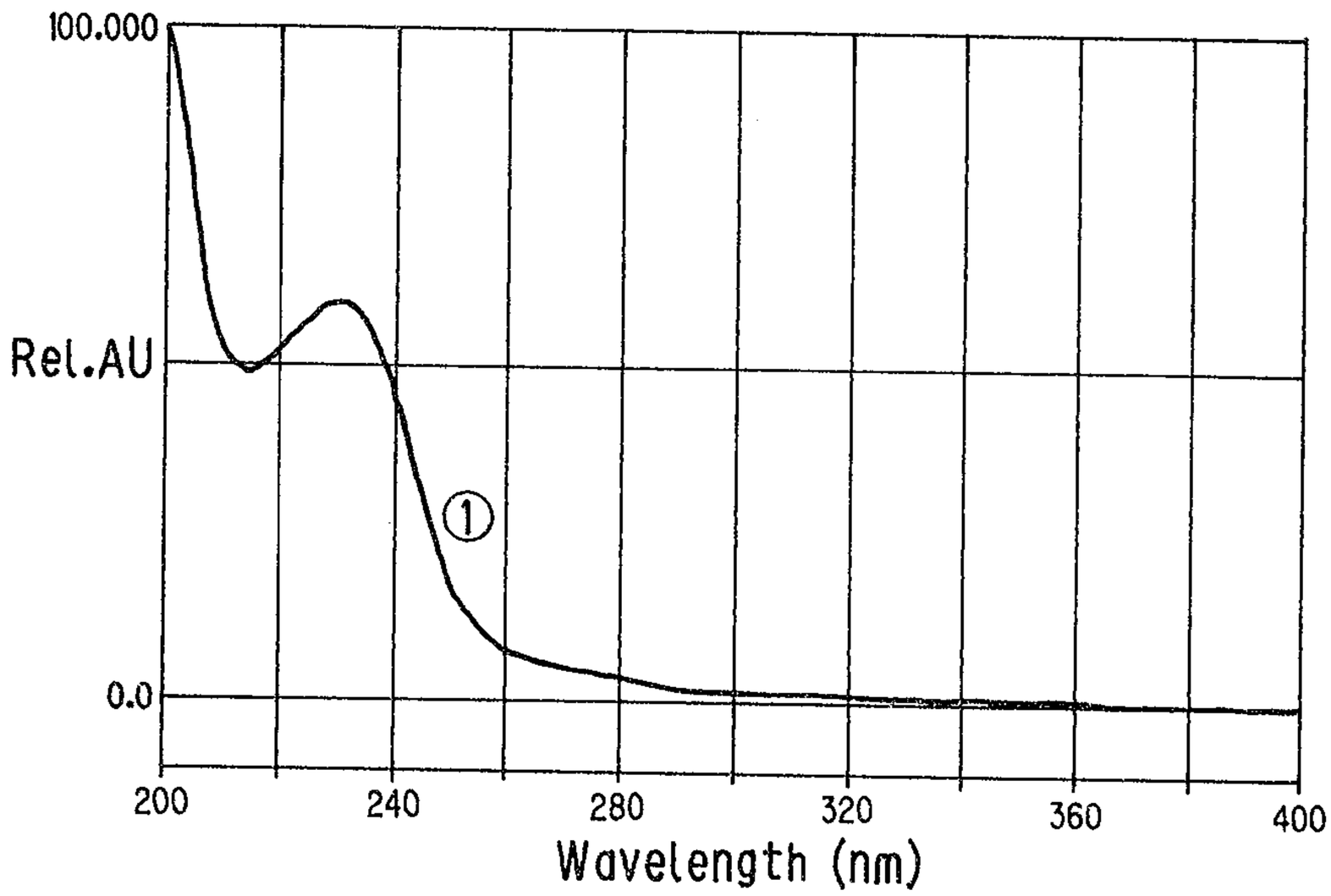

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FIG. 3

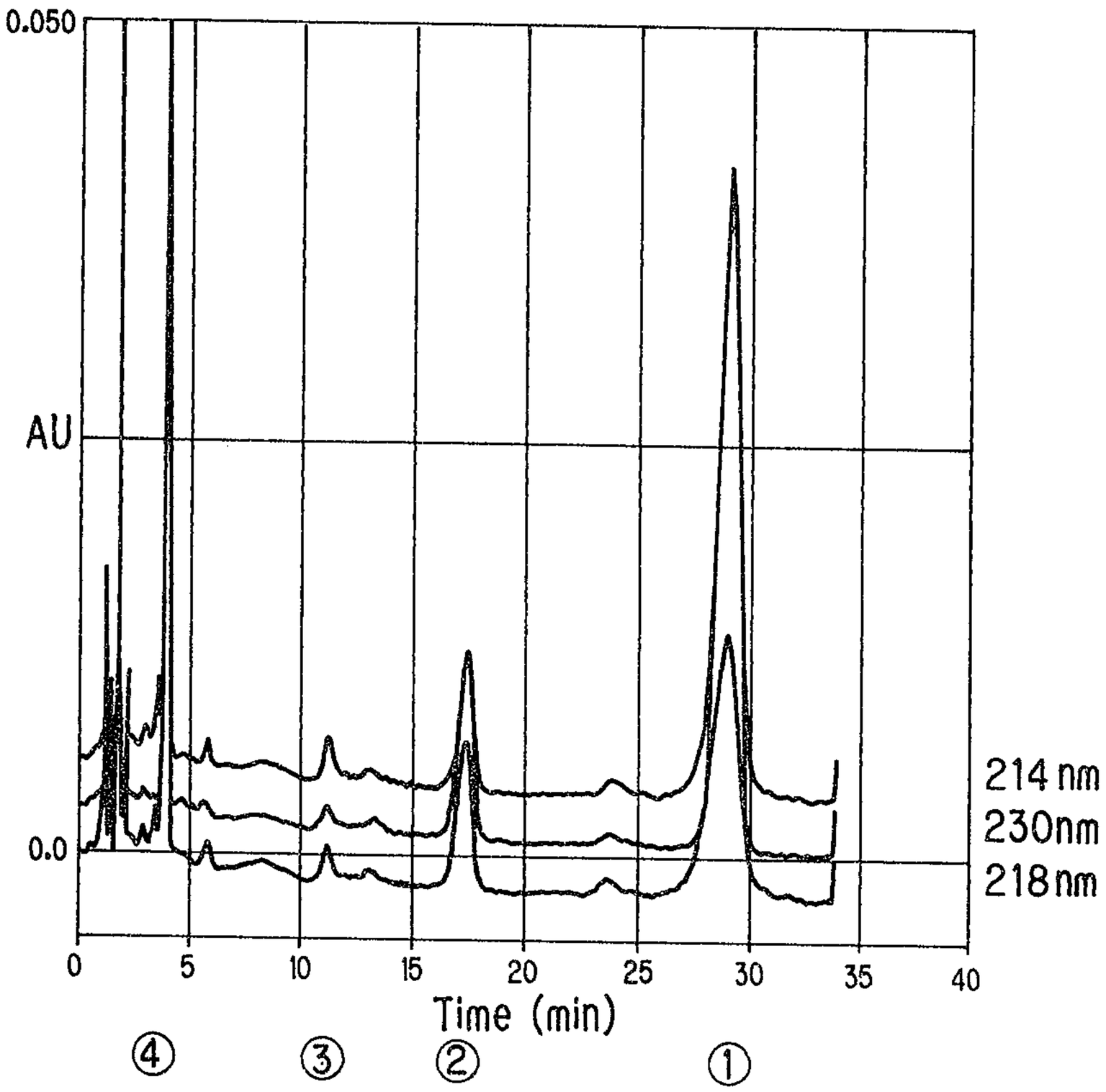

FIG. 4

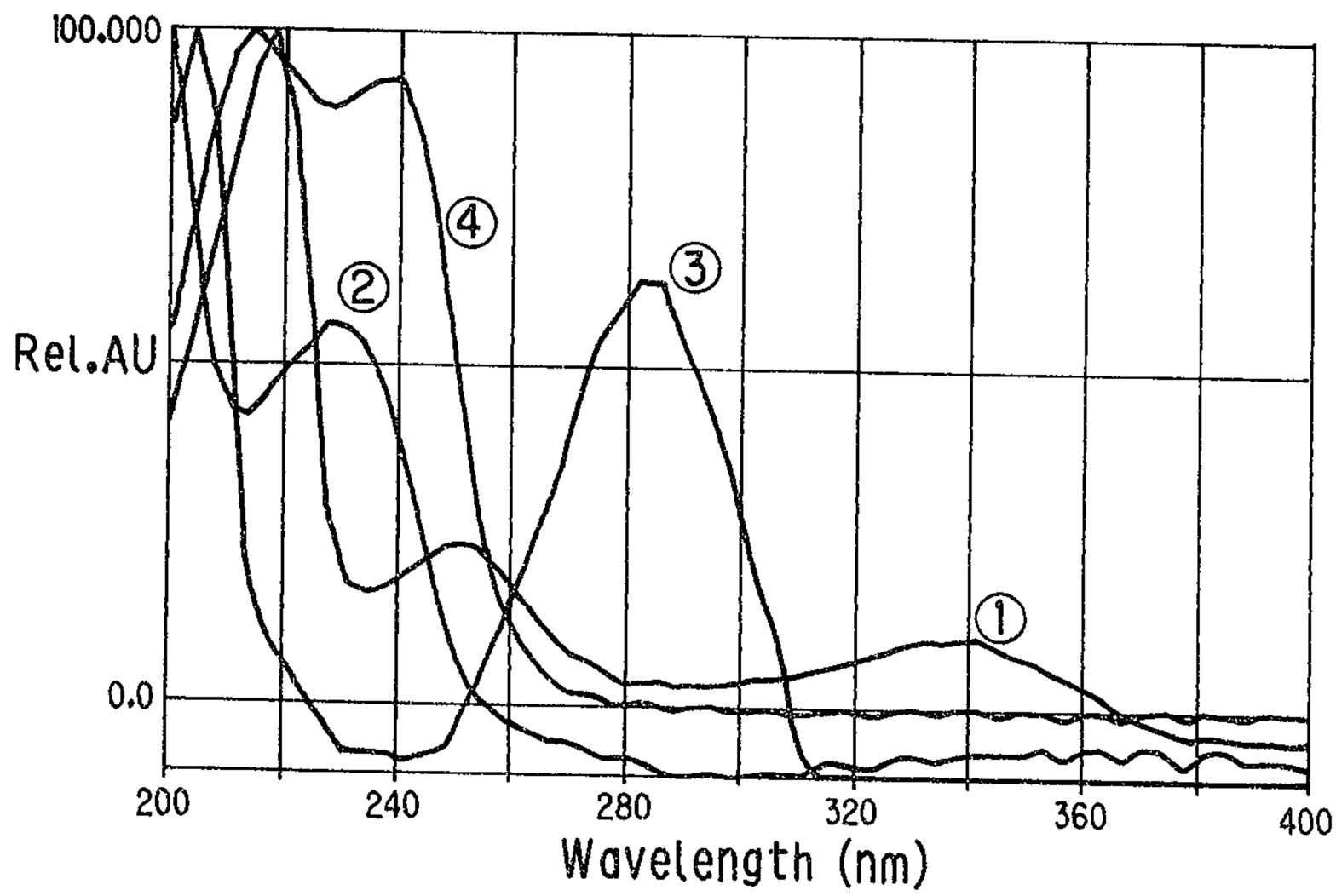

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FIG.5

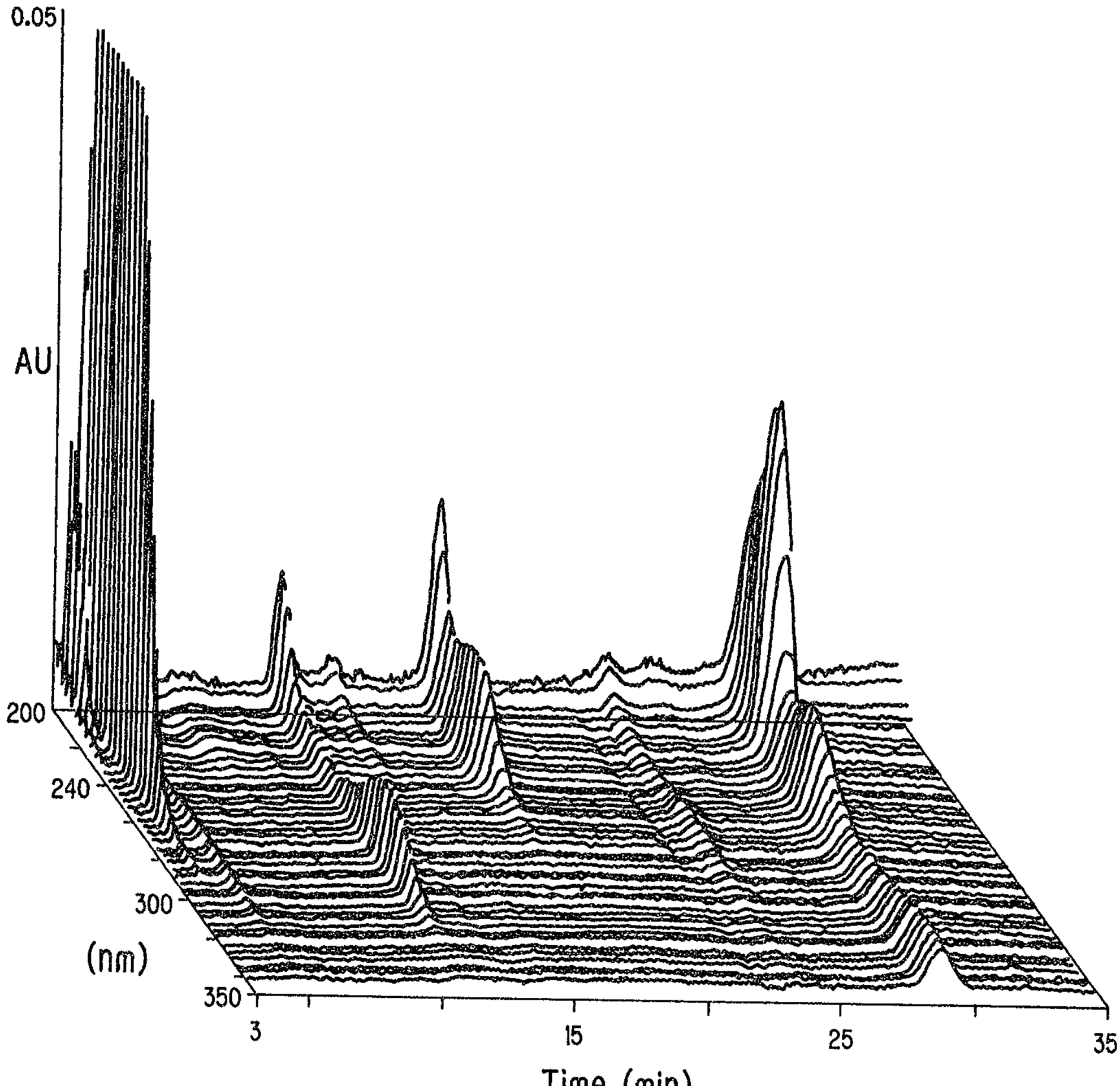

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FIG. 6

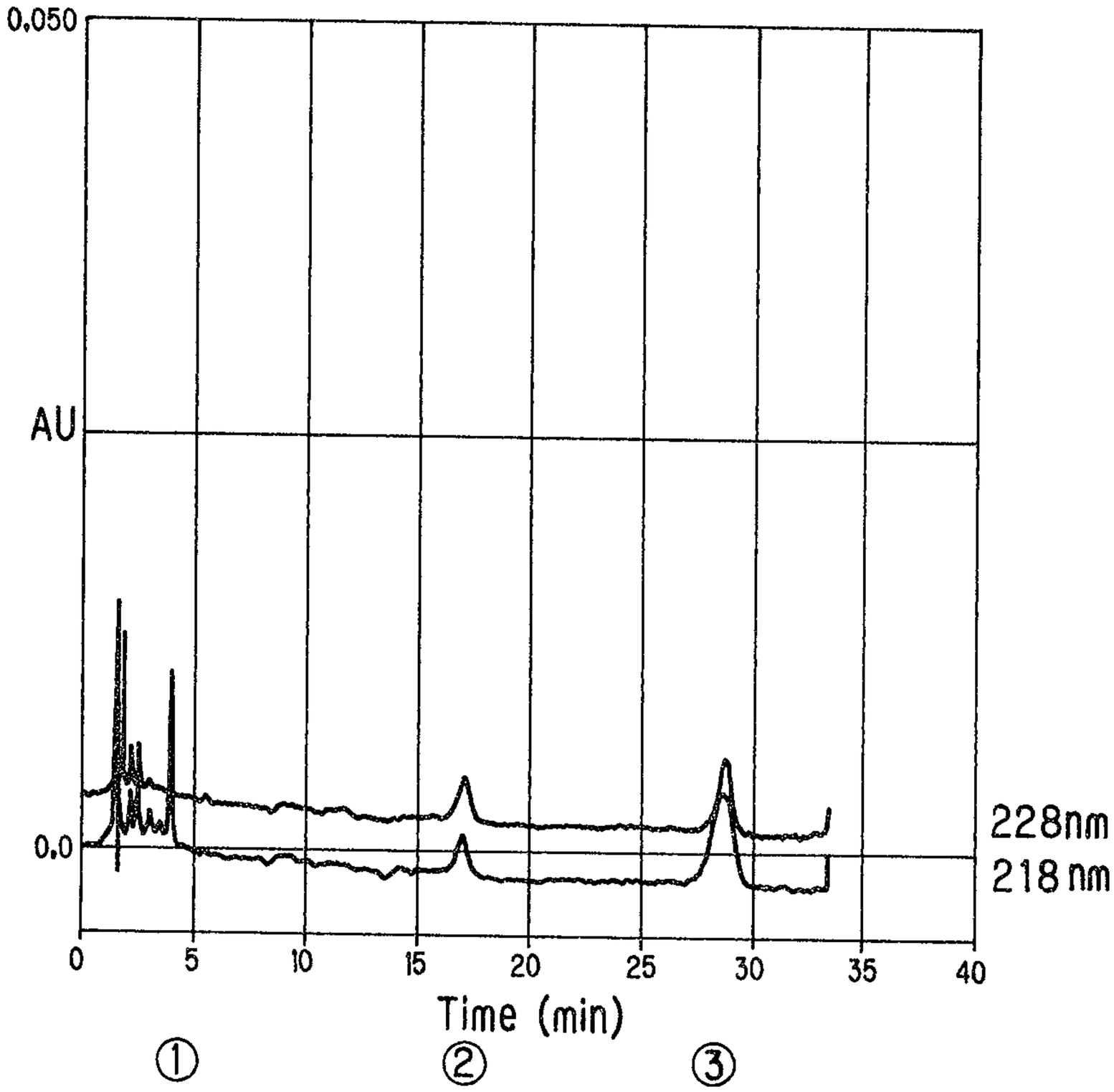

FIG. 7

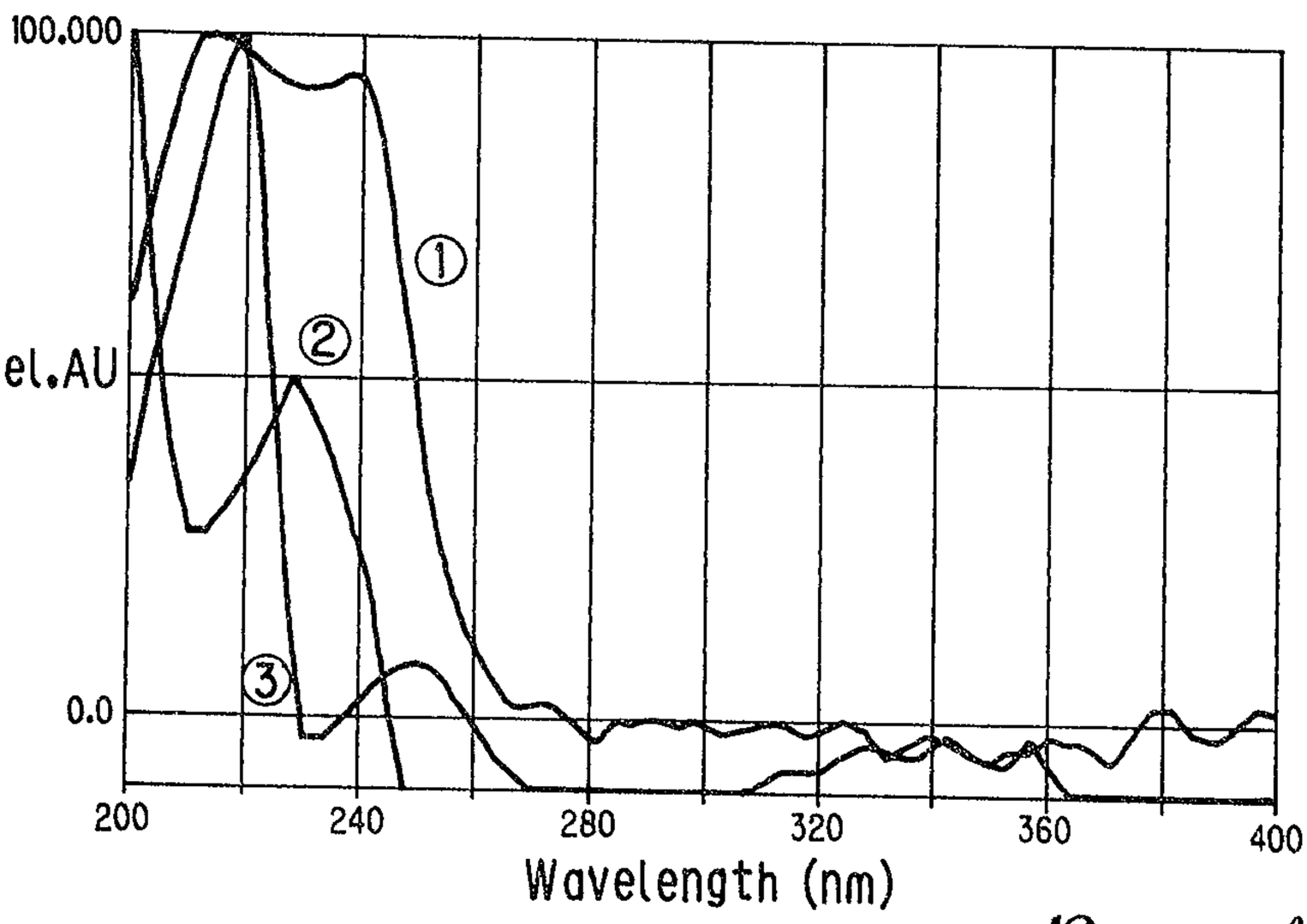

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FIG. 8

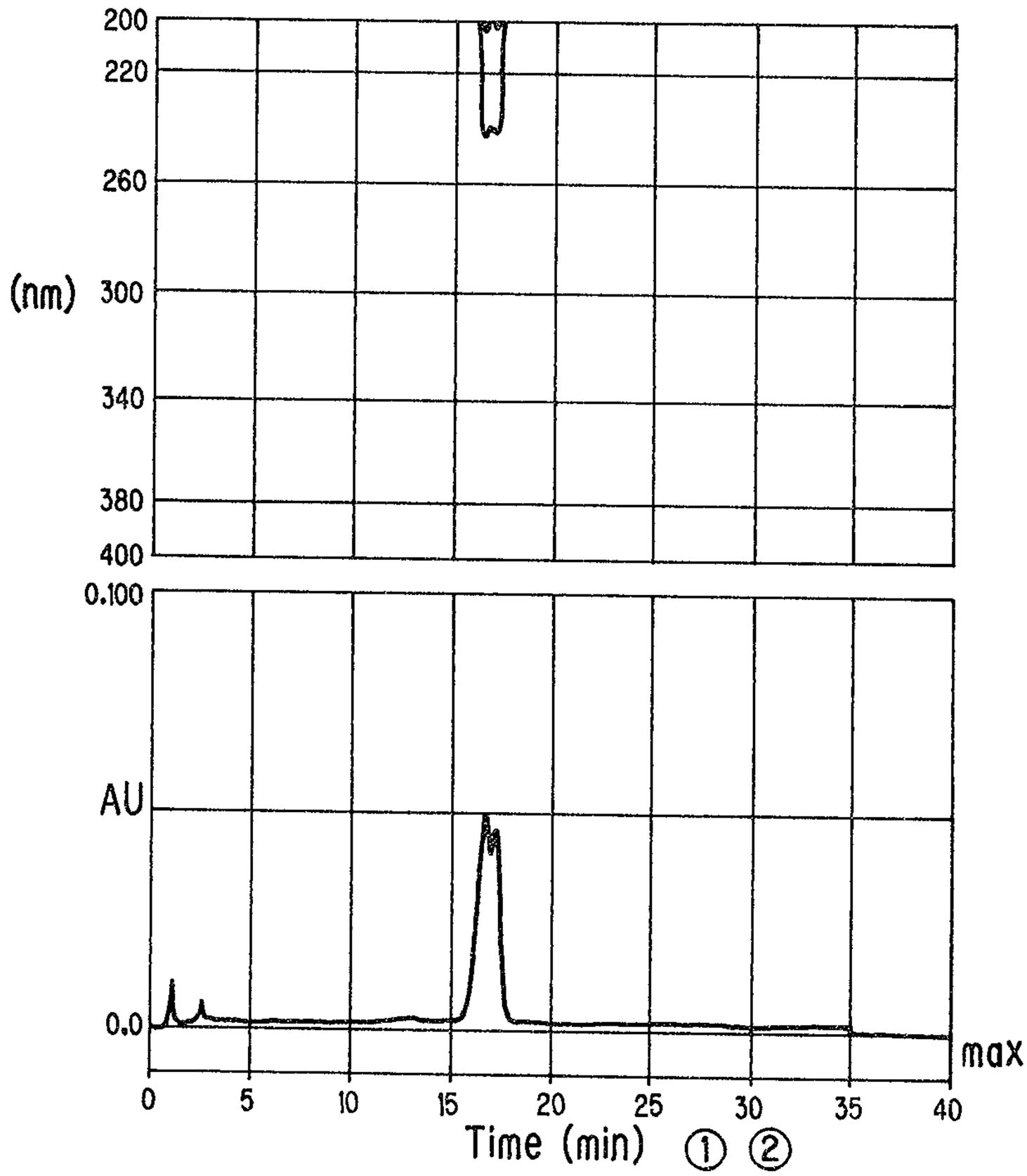
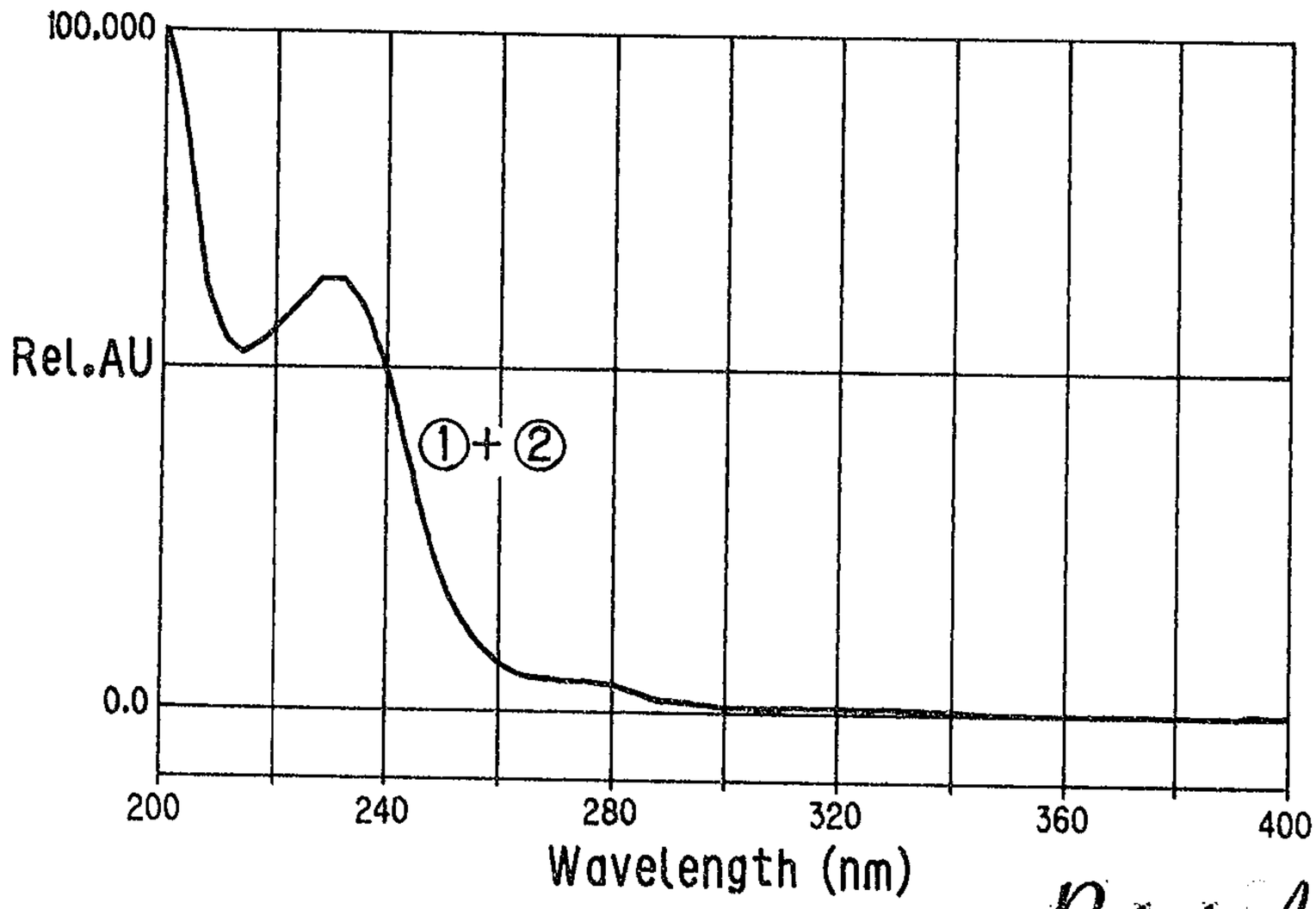


FIG. 9



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