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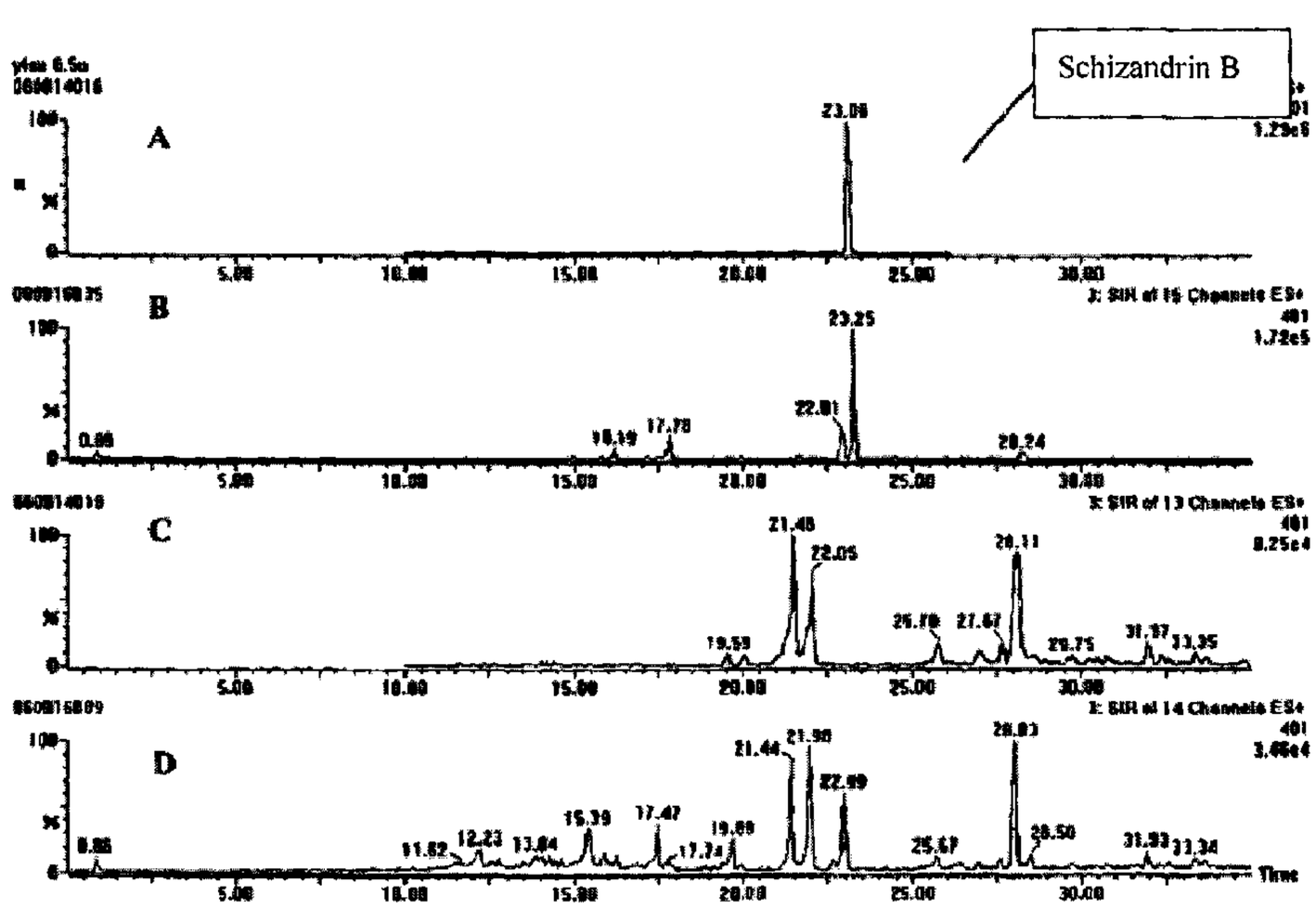
(54) **Titre : PROCEDE DE DETECTION DE SCHIZANDRINE B DANS LE PLASMA SANGUIN APRES ADMINISTRATION DE FUZHENG HUAYU (FZHY)**
(54) **Title: METHOD OF DETECTING BLOOD PLASMA SCHIZANDRIN B AFTER ADMINISTRATION OF FUZHENG HUAYU (FZHY)**



(57) **Abrégé/Abstract:**
A method of detecting blood plasma schizandrin B after administration of Fuzheng Huayu (FZHY) is disclosed. The method includes: (1) extracting schizandrin B from plasma of a mammal administered FZHY by ethyl acetate with the ratio 1:4, whirling 3-

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(57) **Abrégé(suite)/Abstract(continued):**
5mins, centrifuging at 9600rpm for mins, drying and enriching the upper layer at 25- 30 °C, and redissolving with mobile phase; (2) UPLC/MS measuring wherein the UPLC condition comprises an Acquity UPLC BEH C18, 2.1 x 100 mm chromatographic column, mobile phase A comprising water-acetonitrile-formic acid at a ratio (v/v/v) of 95:5:0.1, and mobile phase B comprising acetonitrile-formic acid at a ratio (v/v) of 100:0.1; and the MS condition comprises an electric spraying ion source (ESI) detecting with positive ion mode and scanning at the range of rn/z 150-1200. The method can be used for pharmacokinetics studies of schizandrin B after administration of FZHY.

Abstract

A method of detecting blood plasma schizandrin B after administration of Fuzheng Huayu (FZHY) is disclosed. The method includes: (1) extracting schizandrin B from plasma of a mammal administered FZHY by ethyl acetate with the ratio 1:4, whirling 3-5mins, centrifuging at 9600rpm for mins, drying and enriching the upper layer at 25-30 °C, and redissolving with mobile phase; (2) UPLC/MS measuring wherein the UPLC condition comprises an Acquity UPLC BEH C18, 2.1 x 100 mm chromatographic column, mobile phase A comprising water-acetonitrile-formic acid at a ratio (v/v/v) of 95:5:0.1, and mobile phase B comprising acetonitrile-formic acid at a ratio (v/v) of 100:0.1; and the MS condition comprises an electric spraying ion source (ESI) detecting with positive ion mode and scanning at the range of m/z 150-1200. The method can be used for pharmacokinetics studies of schizandrin B after administration of FZHY.

METHOD OF DETECTING BLOOD PLASMA SCHIZANDRIN B AFTER ADMINISTRATION OF FUZHENG HUAYU (FZHY)

Technical Field

The invention belongs to the field of pharmacokinetics, and particularly involves a method of detecting blood plasma schizandrin B after administration of Fuzheng Huayu (FZHY).

Background Art

FZHY is composed of compound prescriptions including salvia miltiorrhiza, peach kernel, Schisandra chinens etc., which have the effect of curing liver, lung and kidney fibrosis; however, due to a lack of pharmacokinetics research that has been carried out on FZHY, it is not clear about the effective ingredients in vivo, and it is difficult to provide a basis for quality control and guiding clinical rational administration, thus hindering the drug from entering the international market.

So far, no pharmacokinetics research report on FZHY has been found, and there is not any method of detecting schizandrin B after administration of the compound prescription in biological samples (including blood plasma).

Summary of the Invention

There is provided a method for detecting the presence of schizandrin B in the blood plasma of an animal after administering the botanical extract composition Fuzheng Huayu (FZHY) to the animal, comprising the steps of:

(a) collecting the blood plasma from the animal 0.5 hours after administration of FZHY to the animal;

(b) extracting the collected blood plasma with ethyl acetate for 3 minutes to form a blood plasma mixture comprising blood plasma and ethyl acetate at a ratio (v/v) of blood plasma/ethyl acetate equal to 1/4;

(c) centrifuging the blood plasma mixture at 9600 rpm for 10 minutes;

(d) collecting the supernatant after the centrifugation and drying the supernatant at 25-30 °C to form a dried supernatant;

(e) dissolving the dried supernatant in a mobile phase solution comprising a mobile phase A and a mobile phase B to form a blood plasma solution, wherein mobile phase A comprises water, acetonitrile, and formic acid at a ratio (v/v/v) of water/acetonitrile/formic acid equal to 95/5/0.1 and mobile phase B comprises acetonitrile and formic acid at a ratio (v/v) of acetonitrile/formic acid equal to 100/0.1;

(f) preparing conditions for detection by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), wherein the ultra performance liquid chromatography conditions comprise a 2.1 x 100 mm chromatography column, mobile phase A, and mobile phase B; and wherein the mass spectrometry conditions comprise an electrospray ionization ion source in a positive ion mode; and

(g) detecting the presence of schizandrin B in the blood plasma solution using UPLC-ESI-MS/MS under the conditions in step (f) at a mass scan range of m/z 150-800.

Step (g) may be performed under the conditions comprising: a desolvation gas flow of 440 L/h, a desolvation gas temperature of 300 °C, a cone gas flow of 50 L/h, an ion source temperature of 100 °C, a spray capillary voltage of 3800 V, a sampling cone voltage of 30V, an extracting cone voltage of 2.00 V, and a lens voltage of 0.1 V.

There is provided a method for detecting the presence of schizandrin B in the blood plasma of an animal after administering the botanical extract composition Fuzheng Huayu (FZHY) to the animal, comprising the steps of:

(a) collecting blood plasma from the animal 0.5 hours after administration of FZHY to the animal;

(b) extracting the collected blood plasma with ethyl acetate to form a blood plasma mixture;

(c) centrifuging the blood plasma mixture;

(d) collecting and drying the supernatant to form a dried supernatant;

(e) dissolving the dried supernatant in a solution for detection by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), to form a blood plasma solution;

(f) preparing conditions for UPLC-ESI-MS/MS; and

(g) detecting the presence of schizandrin B in the blood plasma solution using UPLC-ESI-MS/MS.

Contents of the Invention

The technical matter to be resolved by this invention is to provide a method of detecting blood plasma schizandrin B after administration of FZHY, and the method is used for pharmacokinetics research, and clarifying the pharmacokinetics rules of blood plasma schizandrin B after administration of FZHY.

The technical problems solved by the invention are achieved through the following technical solutions:

The method of detecting blood plasma schizandrin B after administration of FZHY includes the following steps:

(1) Pretreatment of mammalian plasma samples

a. Mammal plasma containing drugs after administration of FZHY was extracted with ethyl acetate at the volume ratio of 1:4, followed by whirling for 3 to 5 min, centrifuging at 9600 rpm for 10 min; and drying and enriching the supernatant under the condition of 25 to 30 °C;

b. Redissolve eluent with mobile phase and then analyze it with UPLC/MS;

(2) UPLC/MS detection

UPLC condition: chromatographic column: Acquity UPLC BEH C_{18}, 2.1 x 100 mm; mobile phase A: Water-Acetonitrile-Formic acid 95:5:0.1 (v/v/v); mobile phase B: Acetonitrile-Formic acid 100:0.1 (v/v); MS condition: electrospray ionization (ESI) ion source, detection with positive ion mode, scanning at the range of m/z 150 to 1200.

The described step (2) detects with the positive ion mode; the desolvation gas flow is 440L/h, the desolvation gas temperature is 300 °C, the cone gas flow is 50 L/h, the ion source temperature is 100 °C, the spray capillary voltage is 3800 V, the sampling cone

voltage is 30 V, the extracting cone voltage is 2.00 V, the lens voltage is 0.1 V, and the mass scanning at the range of m/z 150 to 1200.

In the process of the liquid-liquid extraction in this invention, the detected components are distributed in two solvents which are immiscible, and by selecting appropriate organic solvents in the appropriate proportion, the non-polar components dissolved in the blood plasma can be extracted into the organic phase, and then after draining and enriching the organic phase, a UPLC system is used to separate analytes from other components, and finally detection is with a mass spectrometric detector.

The ethyl acetate liquid-liquid extraction method adopted by the invention can extract the schizandrin B in the blood plasma, and combined with using a UPLC/MS system to detect, markedly improve the resolution of schizandrin B among other ingredients in the samples, and the analysis method is more sensitive and faster, to facilitate detection of the plasma concentration of schizandrin B in pharmacokinetic research.

Description of Figures

Figure 1. UPLC-MS chromatograms of:

(A) schizandrin B; (B) FZHY; (C) blank plasma; (D) plasma of mammals taken 0.5 hours after drenching with FZHY.

Mode of Carrying out the Invention

Combined with the specific embodiments, further elaboration of this invention is given below. It should be understood that these embodiments are only for description of the present invention and not for the use of limiting the scope of the present invention.

Embodiment 1

A method of detecting blood plasma schizandrin B after administration of FZHY includes:

1. Mammal plasma containing drugs after administration of FZHY was extracted

with ethyl acetate with the volume ratio of 1:4, followed by whirling for 3 min, then centrifuging at 9600 rpm for 10 min, drying and enriching the supernatant under the condition of 25 to 30 °C, redissolving the eluent with mobile phase and then analyzing it with UPLC/MS.

2. UPLC/MS detecting method: the analysis conditions of the UPLC/MS method adopted in this invention: chromatographic column: Acquity UPLC BEH, 2.1 x 100 mm; mobile phase A: Water-Acetonitrile-Formic acid 95:5:0.1 (v/v/v); mobile phase B: Acetonitrile-Formic acid 100:0.1 (v/v), eluting in accordance with the following gradient:

Time（min）	Flow Rate (ml/min)	%A	%B
0	0.300	100	0
5.00	0.300	85.0	15.0
10.00	0.300	70.0	30.0
20.00	0.300	40.0	60.0
30.00	0.300	20.0	80.0
35.00	0.300	20.0	80.0
35.01	0.300	100.0	0.0
38.00	0.300	100.0	0.0

MS conditions: electrospray ionization (ESI) ion source, detecting with positive ion mode; the desolvation gas flow is 440 L/h, the desolvation temperature is 300 °C , the cone gas flow is 50 L/h, the ion source temperature is 100 °C, the spray capillary voltage is 3800 V, the sampling cone voltage is 30V, the extracting cone voltage is 2.00 V, the lens voltage is 0.1 V, and mass scanning at the range of m/z 150 to 1200.

Detection results: Schizandrin B can be detected in the plasma of mammals after drenching with FZHY (see Figure 1).

What is claimed is:

1. A method for detecting the presence of schizandrin B in the blood plasma of a mammal after administering the botanical extract composition Fuzheng Huayu (FZHY) to the mammal, comprising the steps of:

(a) collecting the blood plasma from the mammal 0.5 hours after administration of FZHY to the mammal;

(b) extracting the collected blood plasma with ethyl acetate for 3 minutes to form a blood plasma mixture comprising blood plasma and ethyl acetate at a ratio (v/v) of blood plasma/ethyl acetate equal to 1/4;

(c) centrifuging the blood plasma mixture at 9600 rpm for 10 minutes;

(d) collecting the supernatant after the centrifugation and drying the supernatant at 25-30 °C to form a dried supernatant;

(e) dissolving the dried supernatant in a mobile phase solution comprising a mobile phase A and a mobile phase B to form a blood plasma solution, wherein mobile phase A comprises water, acetonitrile, and formic acid at a ratio (v/v/v) of water/acetonitrile/formic acid equal to 95/5/0.1 and mobile phase B comprises acetonitrile and formic acid at a ratio (v/v) of acetonitrile/formic acid equal to 100/0.1;

(f) preparing conditions for detection by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), wherein the ultra performance liquid chromatography conditions comprise a 2.1 x 100 mm chromatography column, mobile phase A, and mobile phase B; and wherein the mass spectrometry conditions comprise an electrospray ionization ion source in a positive ion mode; and

(g) detecting the presence of schizandrin B in the blood plasma solution using UPLC-ESI-MS/MS under the conditions in step (f) at a mass scan range of m/z 150-800.

2. The method in claim 1 wherein the mass spectrometry step for detecting schizandrin B is performed under the conditions comprising: a desolvation gas flow of 440 L/h, a desolvation gas temperature of 300 °C, a cone gas flow of 50 L/h, an ion source temperature of 100 °C, a spray capillary voltage of 3800 V, a sampling cone voltage of 30V, an extracting cone voltage of 2.00 V, and a lens voltage of 0.1 V.

3. A method for detecting the presence of schizandrin B in the blood plasma of a mammal after administering the botanical extract composition Fuzheng Huayu (FZHY) to the mammal, comprising the steps of:

(a) collecting blood plasma from the mammal 0.5 hours after administration of FZHY to the mammal;

(b) extracting the collected blood plasma with ethyl acetate to form a blood plasma mixture;

(c) centrifuging the blood plasma mixture;

(d) collecting and drying the supernatant to form a dried supernatant;

(e) dissolving the dried supernatant in a solution for detection by ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), to form a blood plasma solution; and

(f) detecting the presence of schizandrin B in the blood plasma solution using UPLC-ESI-MS/MS.

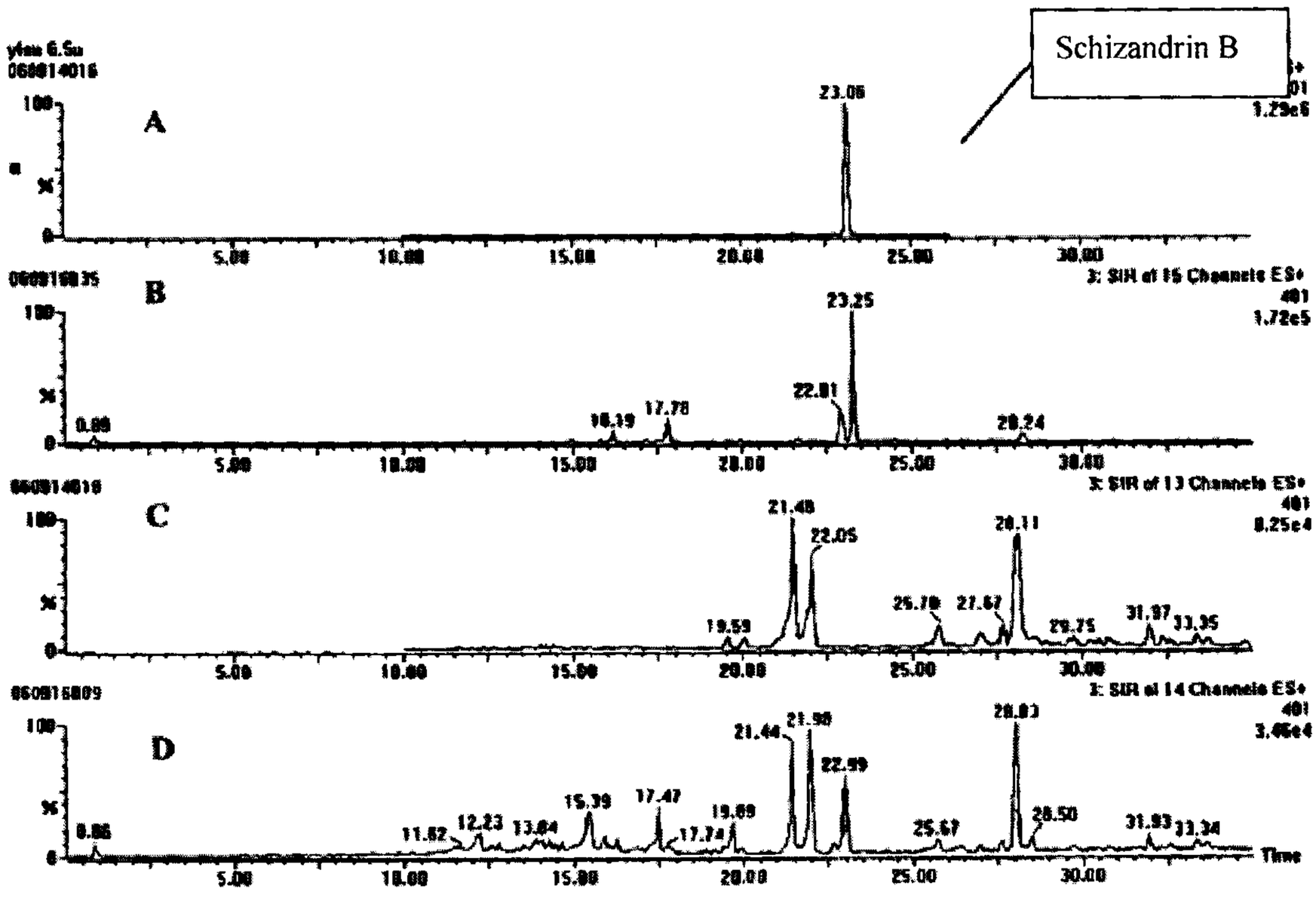
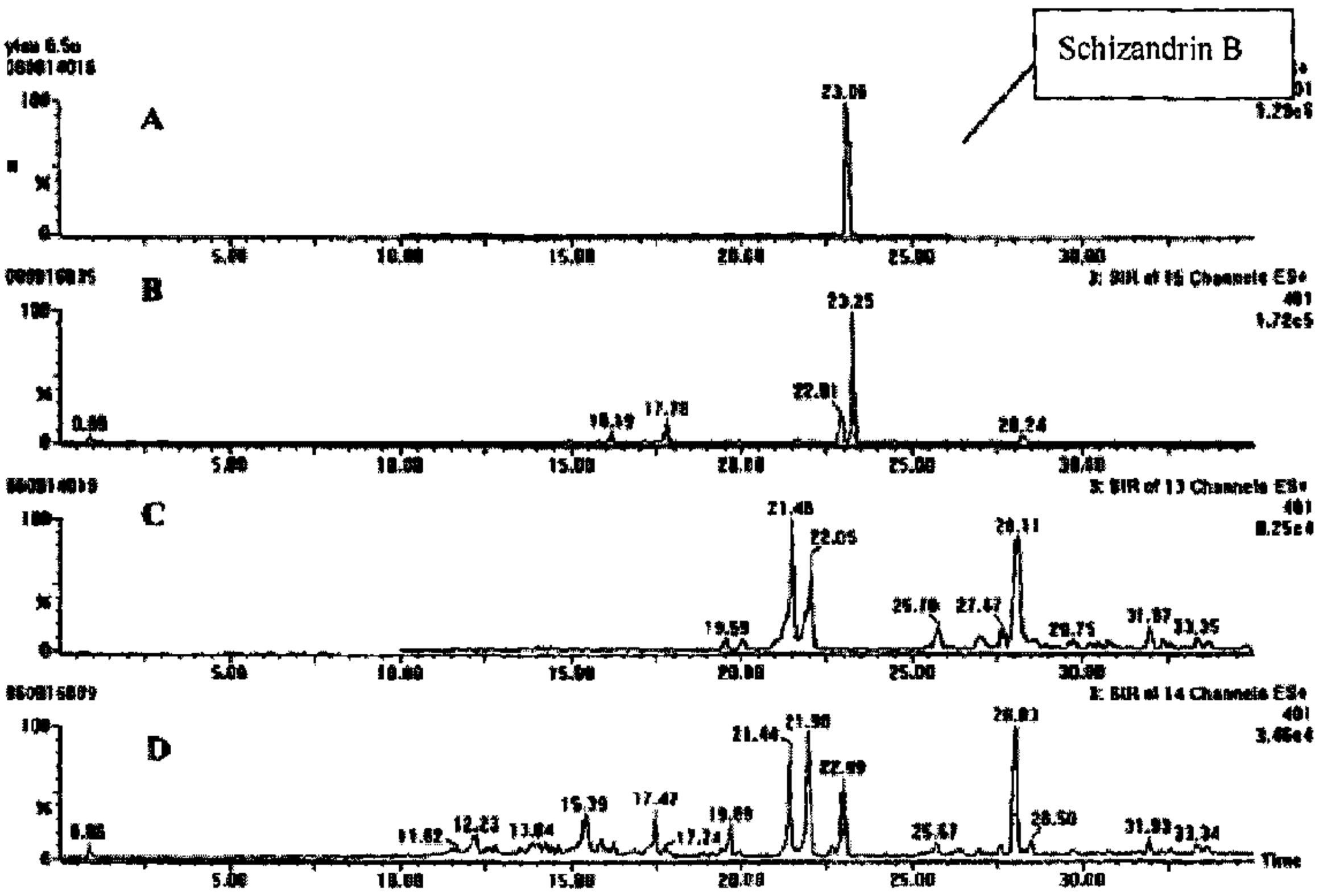

Schizandrin B
A
23.08
B
23.25
22.81
17.78
16.19
0.89
28.24
C
21.48
22.05
28.11
19.59
25.78
27.67
29.75
31.87
33.35
D
21.44
21.90
28.03
22.99
15.39
17.47
19.69
11.62
12.23
13.84
17.74
25.67
28.50
31.83
33.34
0.86
Time

Figure 1


Schizandrin B
A
23.06
B
23.25
22.81
19.19
17.78
0.88
28.24
C
21.48
22.05
20.11
19.59
26.10
27.47
28.75
31.87
33.35
D
21.44
21.90
22.89
28.83
19.39
17.47
19.89
11.82
12.23
13.84
17.24
25.67
28.50
31.83
33.34
0.86
Time