Histamine H1 receptor cell membrane chromatography online high-performance liquid chromatography with mass spectrometry method reveals houttuyfonate as an activator of the histamine H1 receptor

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Abstract

Allergy is an abnormal reaction of the body to an allergen. Histamine is responsible for many of the acute symptoms of allergic diseases. Many of the allergic and inflammatory actions of histamine are mediated by the histamine H1 receptor. In the present study, we established a two-dimensional histamine H1 receptor/cell membrane chromatography with online high-performance liquid chromatography and mass spectrometry method for screening potential histamine-activating components in a traditional Chinese medicine injection. The specification of the method was validated by screening, separating, and identifying a mixed standard solution of diphenhydramine hydrochloride, gefitinib, tamsulosin, and nitrendipine. The Yujin injection, an example of traditional Chinese medicine injection, was screened and potential allergic components acting on histamine H1 receptor was identified. A Ca^{2+} flux assay showed that houttuyfonate and Yujin injection induced calcium release in a dose-dependent manner. This phenomenon suggests that houttuyfonate is an activator of the histamine H1 receptor. The mechanism of houttuyfonate activation involves phosphorylation of the inositol-1,4,5-trisphosphate receptor. In conclusion, this two-dimensional method can rapidly detect and enrich target components isolated from the Yujin injection. This indicates that individuals with an over-expression of the histamine H1 receptor should be aware of possible allergic reactions when receiving the Yujin injection.

Keywords: Adverse drug reactions / Cytosolic free $Ca^{2+/}$ Cell membrane chromatography /Histamine H1 receptor / Houttuyfonate /

Abbreviations: **ADRs**, adverse drug reactions; **TCMI**, traditional Chinese medicine injection; **H_1R**, histamine H1 receptor; **CFDA**, China's state food and drug administration ; **CMC**, cell membrane chromatography; **IP3**, inositol-1,4,5-trisphosphate; **DAG**,

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diacylglycerol; **PKC,** protein kinase C; **MEK**, mitogen-activated protein/extracellular signal regulated kinase; **ERK**, extracellular signal-regulated kinase; **IκB**, inhibitory κB-α;**IKK**, IκB kinase; **NF-κB**, nuclear factor-κB

1 Introduction

Clinical exploration indicates that more than 70% of the adverse drug reactions (ADRs) caused by traditional Chinese medicine injections (TCMIs) are the result of an allergic reaction. Some life-threatening ADRs include anaphylactic shock and fatal anaphylaxis [1].

Histamine is an important chemical messenger mediated by the histamine receptor [2]. Histamine H1 receptors (H_1R), a subtype of histamine receptors, are located in most smooth muscle, endothelial cells, and the central nervous system and are known to mediate various cellular effects involved in immune function and inflammation. The activation of cellular process following histamine binding to the H_1R occurs by phospholipase C-mediated calcium mobilization [3], which induces most allergy-related actions such as smooth muscle contraction, increased vascular permeability, increased secretion of mucus, and increased neuronal firing, etc [4]. Under antigen challenge, immediate hypersensitivity, such as allergic rhinitis, allergic asthma, atopic dermatitis, and allergic conjunctivitis can occur mediated through the H_1R [5].

The Yujin injection is composed of volatile oils extracted from the Chinese medicinal plants houttuynia cordata (*Houttuynia cordata Thunb.*) and honeysuckle (*Lonicera japonica Thunb.*) using steam distillation. It is widely used for the treatment of upper respiratory infections, bronchopneumonia, and viral pneumonia, and other allergy symptoms. The common side effects include local pain, redness, itchy skin, and rash, etc. A review of clinical trials indicates that adverse reactions occur frequently in recipients of the Yujin injection; severe allergic reaction was one of the observed clinical manifestations[6–7]. In 2006, China's State Food and Drug Administration (CFDA) alerted healthcare professionals to stop using seven kinds of herbal medicine injections containing the herb *H. cordata*, such as the Yujin injection [www.sda.gov.cn/WS01/CL0087/14804.html; www.sda.gov.cn/yxc/1.htm]. Reports of the Chinese National Center for Adverse Drug Reaction Monitoring revealed that

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the Yujin injection was associated with a number of serious allergic reactions such as allergic shock, systemic allergic reaction, chest congestion, palpitations, breathing difficulty, and severe drug eruption after intravenous administration. The ADRs induced by the Yujin injection are possibly attributed to its complex components and various mechanisms of activity. Because the mechanism of the Yujin injection has not yet been fully elucidated, a powerful method of screening for the allergic components in the Yujin injection and accurate and quick drug safety evaluation for predicting the allergenicity of such herbal injections is needed urgently.

Cell membrane chromatography (CMC) is a type of biological affinity chromatography proposed in 1996, in which the stationary phase has certain cell membrane receptors that can easily detect the interaction between drugs and membrane receptors [8–10]. In our laboratory, we have successfully constructed cells highly expressing the H_1R named HEK293/H_1R cells. Cells with a high expression of the H_1R can be used to screen compounds acting on the inflammation-associated H_1R in TCMIs.

In the present study, we developed a 2-D H_1R-CMC–HPLC–MS system for screening for the allergic components in the Yujin injection. In addition, the concentration of cytosolic free Ca^{2+} and the level of phosphorylation of inositol-1,4,5-trisphosphate receptor (IP3R) were measured to detect the allergenicity of the screened components.

2 Materials and methods

2.1 Drugs and materials

Diphenhydramine hydrochloride (Lot Number: 100066-200807), and nitrendipine (Lot Number: 100585-200602) were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Sodium houttuyfonate (Lot Number: HX0120510, purity>99%) was from Baoji Haoxiang Bio-technology. Tamsulosin hydrochloride (purity>98%) was from Wuhan Chifei Chemical (Wuhan, China). Gefitinib (purity>99.2%) was from Nanjing Ange Pharmaceutical (Nanjing, China). The Yujin injection (Lot Number: 12100046) was from Shaanxi Pharmaceutical Holding Group Shan Hai Dan Pharmaceutical (Xi'an, China). Methanol (HPLC grade) was obtained from Fisher

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Scientific (Pittsburgh, USA). Macroporous spherical silica gel (5 μm, 100–200 mesh) was from Meigao Chemical (Qingdao, China). Ammoniumacetate was from Fuchen Chemical Reagent Factory (Tianjin, China). All aqueous solutions were prepared using fresh threefold-distilled water.

2.2 Preparation of standard solutions and samples

Standard stock solutions (1 mg/mL) of sodium houttuyfonate and diphenhydramine hydrochloride were separately prepared in methanol. A sample of the Yujin injection was diluted to 1 mg/mL with the mobile phase solution. All the solutions were stored in the dark at 4°C and freshly prepared every week. Working solutions (0.01 mg/mL) were diluted with the mobile phase solution for use.

2.3 Instrumentation

Chromatographic analysis was performed using a 2-D system that consisted of three LC-20AD pumps, a DGU-20A3 degasser, a CTO-20AC column oven, a SIL-20A autosampler, a SPD-20A UV/VIS detector, a SPD-M20A diode array detector (DAD), an LC/MS-2010EV mass spectrometer and LC/MS solution workstation (Shimadzu, Kyoto, Japan).

A ten-port two-position valve (VICIAG 10G-0911V, Valco Instrument, TX, USA) was used as the column switcher to switch among the 2-D systems. By means of the switcher, the H_1R-CMC model (1st D) was combined with the HPLC–MS system (2nd D) in an online mode. An H_1R-CMC column (10.0 mm×2.0 mm i.d.) was used as the first-dimensional column and a Shim-pack octadecylsilyl (ODS) column (150 mm×2.0 mm i.d., 5 μm, Shimadzu) served as the second-dimension column. Two Shim-pack octadecylsilyl pre-columns (10.0 mm×2.0 mm i.d., 5 μm, Shimadzu) were used as the enrichment columns.

2.4 Preparation of the H_1R-CMC column

Cell culture and preparation of the H_1R-CMC columns were conducted as follows. Overexpressed H_1R cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, MD, USA) supplemented with 10% v/v fetal bovine serum (Hyclone, UT, USA). Cells were cultured in a 5% CO_2 and 95% humidified air incubator at 37°C. Stock cultures were passed weekly in the presence of G418 (0.3 g/L) in medium to maintain the high expression of the H_1R. Cultured HEK293 H_1R cells (7×10^6) were washed three times with

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cold phosphate-buffered saline solution by centrifuging at 400 *g* for 10 min. Cells were resuspended in hypotonic Tris-HCl (50 mM, pH 7.4) and were supersonically ruptured for 3 min in ice. The resulting homogenate was centrifuged at 1000 *g* for 10 min. The supernatant was centrifuged at 12,000 g for 20 min. The H_1R membrane suspension was obtained after suspending the precipitation in 10 mL Tris-HCl (50 mM, pH7.4). All procedures were performed at 4°C.

According to the previously reported method [9], the H_1R cell membrane stationary phase (CMSP) was prepared as follows: 0.05 g silica was activated at 105°C for 30 min and homogenized with the H_1R membrane suspension under a vacuum. The mixture was agitated for 60 min and left to stand overnight at 4°C. After washing twice with cold physiological saline, the H_1R CMSP was packed into the column (10×2.0 mm i.d.) using a wet-packing method (10 MPa, 3 min) to yield a H_1R-CMC column.

2.5 Reproducibility and lifetime of the H_1R-CMC column

The reproducibility of the H_1R column was studied respectively by repeated injections of diphenhydramine hydrochloride (0.01 mg/mL). The retention time was used as an indicator. After attaching to the HPLC instrument, the column was equilibrated for about 60 min with aqueous ammonium acetate buffer solution (10 mM, pH7.4). For one H_1R-CMC column or for different H_1R-CMC columns, reproducibility was determined using repeated injections of 10 μL 0.01 mg/mL diphenhydramine hydrochloride. Concurrently, the lifetime of the H_1R-CMC column was also investigated using repeated injections of 10 μL of diphenhydramine hydrochloride (0.01 mg/mL).

All of the chromatographic conditions were identical: the mobile phase used was aqueous ammonium acetate buffer solution (10 mmol/L, pH7.4) with a flow rate of 0.2 mL/min. Data acquisition was executed using an UV detector and the detection wavelength was 254 nm. The column temperature was 37°C.

2.6 H_1R-CMC–HPLC–MS method

A ten-port two-position switch valve was used to combine the H_1R-CMC column with the HPLC–MS system (Figure 1). The preparation of this online system followed previous studies [11, 12]. Each sample was injected into the H_1R-CMC model (1^{st} D). Any retention fraction in the H_1R-CMC model was enriched onto an ODS pre-column (PC1 or PC2), and

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then the enriched fraction was eluted into the HPLC–MS system (2nd D) for analysis.

The chromatographic conditions of the first-dimensional H_1R-CMC were as follows: An H_1R-CMC column was used. For diphenhydramine hydrochloride, the mobile phase (M1) was aqueous ammonium acetate buffer solution (10 mM, pH 7.4). For sodium houttuyfonate and the Yujin injection, the mobile phase (M1) was water. The flow rate was 0.2 mL/min. The detection wavelength of the UV detector for diphenhydramine hydrochloride was 254 nm, while for sodium houttuyfonate and Yujin injection it was 284 nm. For the HPLC–MS a VP-ODS column was used: the mobile phase was methanol/water-0.1 mol/L aqueous ammonium acetate buffer solution (pH 7.4) (55:45, v/v) for diphenhydramine hydrochloride; the mobile phase was methanol/water-0.1 M aqueous ammonium acetate buffer solution (pH 7.4) (70:30, v/v) for the Yujin injection; the mobile phase was methanol/water-10 mM aqueous ammonium acetate buffer solution (pH 7.4) (65:35, v/v) for sodium houttuyfonate. The flow rate was 0.2 mL/min. The column temperature was kept at 37°C, with a DAD. The MS conditions were as follows: ionization mode, ESI; nebulizer gas, N_2 (purity 99.999%), 1.5 L/min; drying gas, N_2 (purity 99.999%) with a pressure of 0.1 MPa; interface temperature, 250°C; heat block temperature, 200°C; curved desolvation line (CDL) temperature, 230°C; CDL voltage, 10 V; detector voltage, 1.5 kV; positive and negative ionization mode, scanning *m/z* 50–800.

2.7 Validation of the H_1R-CMC–HPLC–MS method

The capability of the H_1R-CMC–LC–MS method was validated. Diphenhydramine hydrochloride (acting on the H_1R) was applied as the positive control and tamsulosin (acting on the α_{1A} receptor), nitrendipine (acting on L-type calcium channels), and gefitinib (acting on the epidermal growth factor receptor) as negative controls.

When 10 μL of a mixed standard solution of tamsulosin (0.01 mg/mL), diphenhydramine hydrochloride (0.01 mg/mL), nitrendipine (0.01 mg/mL), and gefitinib (0.01 mg/mL) was injected into the online system, the non-retained and retained fractions on the H_1R-CMC column were captured, concentrated online, and sequentially switched into the 2-D HPLC–MS system for analysis. In brief, the chromatographic conditions were as follows: the mobile phase was aqueous ammonium acetate buffer solution (10 mmol/L, pH 7.4). The flow rate was 0.2 mL/min. The column temperature was 37°C. The detection wavelength of

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the UV detector was 254 nm. The mobile phase (M2) was methanol/water-0.1 mol/L aqueous ammonium acetate buffer solution (pH 7.4) (55: 45, v/v). The flow rate was 0.2 mL/min. The column temperature was kept at 37°C, with a DAD detector. The MS conditions were the same as those described in Section 2.6.

2.8 Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]c$) measurements

Fluo-3/AM is a Ca^{2+}-reactive fluorescent probe that is usually used to detected the $[Ca^{2+}]c$ [13]. Cells (2×10^6 cells), cultured in 6 cm vessels, were incubated with 4 μM Fluo-3/AM-containing DMEM medium for 30 min at 37°C. At the same time, with the purpose of increasing the cell membrane permeability, 20% pluronic F-127 was added. Subsequently, 5 times the volume of Ca^{2+}-free physiological salt solution (PSS) supplemented with 1% v/v defined fetal bovine serum was added. After incubation for a further 45 min at 37°C, the cells were washed and resuspended in Ca^{2+}-free PSS. After stimulation with various volumes of Yujin injection or various concentrations of sodium houttuyfonate, cells were immediately (within 30 s) excited at 488 nm by the microplate fluorimeter. And the intracellular fluorescence intensity was detected instantly at 527 nm.

2.9 Immunoblotting analysis

All data were analyzed using GraphPad Prism 5.0 software. After incubation with different concentrations of sodium houttuyfonate for 5 min, the H_1R cells were incubated with cold radio immunoprecipitation assay (RIPA) lysis buffer (50 mM Tris (pH, 7.4), 150 mM NaCl, 1 mM of EDTA, 1 mM Na_3VO_4, 1% sodium deoxycholate, 1% Triton X-100, 0.5 μg/mL leupeptin, 25 mM β-glycerophosphate, and 0.1% SDS supplemented with protease inhibitor cocktail, and phosphatase inhibitor cocktail (Roche Applied Science, Basle, Switzerland) on ice for 30 min. The concentration of extracting protein was detected using a bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, MA, USA). Next, 25 μg proteins per lane were resolved using a 6% SDS-PAGE gel and transferred to a nitrocellulose (NC) membrane (GE Healthcare, NJ, USA). After blocking with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) buffer for 2 h, the membranes were probed with primary antibodies (anti-IP3R (1:200), anti-p-IP3R (1:1000), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 1:100) overnight at 4°C followed by incubation with their respective secondary antibodies conjugated with horseradish

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peroxidase for 2 h at room temperature. Fluorescence signals were detected using an enhanced chemiluminescence (ECL) detection system (Pierce, Rockford, USA) and analyzed with the Quantity one® 1-D analysis software (version 4.4, Bio-Rad). GAPDH was used as reference to determine the relative protein level.

Statistical analysis

The results were expressed as mean ± SD. Statistical differences were assessed using Dunnett's *t* test. The statistical significance of a result was determined to be $p < 0.05$.

3 Results and discussion

3.1 Reproducibility and lifetime of the H_1R-CMC column

Diphenhydramine hydrochloride can act on the H_1R specifically [14]. Therefore, we chose diphenhydramine hydrochloride as a positive drug to study the characteristics of the H_1R-CMC column (Table 1). The reproducibility of a single H_1R-CMC column was assessed by injecting a standard solution of diphenhydramine hydrochloride (0.01 mg/mL) five times. The RSD of the retention times and peak areas of diphenhydramine hydrochloride on a single H_1R-CMC column were 1.72% (n=5) and 7.78% (n=5), respectively. The reproducibility of different H_1R-CMC columns was determined by injecting the standard solution of diphenhydramine hydrochloride (0.01 mg/mL) into three different H_1R-CMC columns. The RSD of the retention time on the three different H_1R-CMC columns was 7.40% (n=3). Concurrently, we also explored the lifetime of the H_1R-CMC columns since the H_1R-CMC column was biologically active. By repeatedly injecting diphenhydramine hydrochloride into a H_1R-CMC column, the retention time decreased merely 11.67% (8.621–7.615 min) during 72 h application. Therefore, to ensure activity retention, all of the H_1R-CMC columns below were used for only 72 h after they were packed. It was confirmed that the H_1R-CMC columns developed in this study met the requirements for screening active components acting on the H_1R.

3.2 Checking the H_1R-CMC–HPLC–MS system

A mixed standards solution of tamsulosin (acting on the α_{1A} receptor), nitrendipine (acting on L-type calcium channels), gefitinib (acting on the epidermal growth factor

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receptor), and diphenhydramine hydrochloride was used to validate the specific selectivity of the H_1R-CMC–HPLC–MS system (Figure 2). Among the four standard drugs, diphenhydramine hydrochloride acted specifically on the H_1R.

As shown in Figure 2, two fractions (R0, representing non-retained components; R1, representing retained components indicated between the dotted lines) were sequentially enriched by two ODS pre-columns, which were loaded with C_{18} and had a greater retentive ability than the H_1R-CMC column and could be easily washed out. After switching of the two-position ten-port valve, the two fractions were eluted from each enriching pre-column alternatively into the second-dimensional VP-ODS column for chromatographic separation and MS identification. The chromatograms and mass-spectrum of R1 and R0 are shown in Figure 2C and D. Figure 2C confirmed that R1 was diphenhydramine hydrochloride (*m*/*z* 256), which had the same retention characteristics as that in the direct LC–MS chromatogram of the mixed standards solution (Figure 2B). The results demonstrated that, from the mixed standards solution, only diphenhydramine hydrochloride could be specifically retained by the H_1R-CMC system and further analyzed using the HPLC–MS system. This indicated that the H_1R-CMC–HPLC–MS method developed in this study was suitable for screening active small molecular components acting on the H_1R from the complex samples.

3.3 Applications

In this study, we used the coupled H_1R-CMC–HPLC–MS system to screen for active components from the Yujin injection that acted on the H_1R. As shown in Figure 3, one retention fraction (R) was detected on the H_1R-CMC column (Figure 3A). After enrichment by the enriching pre-column and transfer into the 2-D HPLC–MS system, the retention fraction R was further analyzed and identified. Additional mass spectrum analysis showed the main component of retention fraction R was likely to be sodium houttuyfonate (*m*/*z* 198) (Figure 3B). Figure 3C is the HPLC–MS chromatogram of the direct injection of the Yujin injection.

To further confirm the above results, standard sodium houttuyfonate was investigated (Figure 4). After injection into the H_1R-CMC column (Figure 4A), the standard solution of sodium houttuyfonate had a distinct retention (R1), which had a similar retention time to that of the retention fraction (R) of the Yujin injection, shown in Figure 3A. It was confirmed that

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sodium houttuyfonate could be retained on the H_1R-CMC column. After transfer into the second dimension system, the retention (R1) was analyzed using HPLC–MS and further mass spectrum analysis (Figure 4B). Results revealed that the HPLC–MS chromatogram of standard sodium houttuyfonate (Figure 4B) exhibited one similar major chromatographic peak to that of the HPLC–MS chromatogram of fraction R (Figure 3B). The major chromatographic peak in Figure 4B was also thought to be sodium houttuyfonate (*m*/*z* 198), the same as in Figure 3B. Based on the results, sodium houttuyfonate was identified as a specific component from the Yujin injection that could interact with the H_1R.

3.4 Effect of sodium houttuyfonate on cytosolic free Ca^{2+} concentration

In the present study, the H_1R-CMC–HPLC–MS system screened the active component sodium houttuyfonate from the Yujin injection. Subsequently, we further explored the pharmacological activity of sodium houttuyfonate in the activation of the H_1R.

H_1R is an example of a G-protein coupled receptor. When activated, the H_1R results in an increase in cytosolic free Ca^{2+}, which induces smooth muscle contraction and vascular permeability [5]. Activation of the H_1R induces the activation of extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB) cascades, which are triggered through Ca^{2+}-dependent protein kinase C (PKC) stimulation. These cascades then induce the synthesis of several proinflammatory cytokines, such as interleukin (IL)-6 and IL-8, which are associated with allergic responses [15]. The concentration of cytosolic free Ca^{2+} is increased not only by enhanced penetrability of the cell membrane, which can increase extracellular free Ca^{2+} influx, but through promotion of Ca^{2+} release from the endoplasmic reticulum [16]. The enhancement of cytosolic free Ca^{2+} could represent the level of the activation of H_1R.

Figure 5A shows the effects of different volumes of Yujin injection on changes in the concentration of cytosolic free Ca^{2+} in H_1R-HEK293 cells. As shown in Figure 5A, in the presence of Yujin injection at volumes of 0, 0.064, 0.32, 1.6, 8, 40, and 200 μL, an obvious rise of fluorescence was observed. It was confirmed that the Yujin injection could increase cytosolic free [Ca^{2+}] influx. To confirm whether the screened components from the Yujin injection have the same pharmacological effects, H_1R-HEK293 cells were treated with serial concentrations of 0, 3.9, 15.6, 62.5, 250, and 2000 μg/mL of sodium houttuyfonate (Figure 5B). The resulting fluorescence increased in a dose-dependent manner. Thus, sodium

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houttuyfonate, screened from the Yujin injection, had a high potency in the activation of the H_1R (Figure 5B). Activation of the H_1R occurred at the higher concentration, which was in agreement with the screening results shown in Figure 3.

3.5 Effect of sodium houttuyfonate on p-IP3R protein expression

Previous studies have shown that activation of the H_1R induces the activation of phospholipase C and generates inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) [17, 18]. The activation of IP3 induces the release of Ca^{2+} from calcium stores, and subsequently activates store-operated calcium channels, which increases the concentration of cytosolic free Ca^{2+}. Ultimately, this pathway leads to the mediation of inflammatory responses via effects on the production of proinflammatory cytokines through PKC/Raf/mitogen-activated protein extracellular signal regulated kinase kinase (MEK)/ERK and PKC/IκB kinase (IKK)/inhibitory κB-α (IκB)/NF-κB p65 signal cascades [16], and induces smooth muscle contraction and vascular permeability.

The p-IP3R expression level was measured. After treatment with different concentrations of sodium houttuyfonate, the p-IP3R levels of H_1R-HEK293 cells increased in a dose-dependent manner (Figure 6). Since it was confirmed that sodium houttuyfonate increased the concentration of cytosolic free Ca^{2+} (Figure 5B) and induced the activation of IP3R (Figure 6) in H_1R-HEK293 cells, we hypothesized that sodium houttuyfonate might associate with the H_1R-IP3R-Ca^{2+} pathway. Based on these results, we proposed that sodium houttuyfonate is an allergic agent, which can induce the activation of the H_1R and may affect the occurrence of allergic reactions.

The herbal injection Yujin consists of two herbs, houttuynia cordata and honey suckle, and it displays heat-clearing and detoxifying efficacy. *H. cordata*, a fishy-smelling herbaceous perennial plant, is an ancient herbal medicine widely used in traditional Chinese medicine for treating pneumonia, bronchitis, dysentery, urinary tract infections, and poisonous snakebite. A famous application of *H. cordata* was its use by Chinese scientists to tackle severe acute respiratory syndrome (SARS) because of its ability to relieve abnormal lung symptoms [19]. Sodium houttuyfonate is one of the most important active ingredients of *H. cordata* and also the main component of the herbal injection Yujin. However, based on the above studies, we should take into consideration the concentration of sodium houttuyfonate

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to reduce the risk of potential allergic effects during its use.

4 Conclusions

A 2-D H_1R-CMC–HPLC–MS method was established and applied to screen potential active components from the Yujin injection that might interact with the H_1R. Sodium houttuyfonate, one of the efficacious components of the Yujin injection, was identified as the active component, using this system. The measurement of cytosolic free [Ca^{2+}] and determination of p-IP3R expression confirmed that the screened sodium houttuyfonate could activate the H_1R, which might affect the occurrence of allergic reactions. This method has practical application in the screening of active components from other herbal medicine injections that might interact with the H_1R, to better assess the safety of traditional Chinese medicine treatment.

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Table 1. Reproducibility and lifetime of the H_1R-CMC column.

		RSD of retention time (n=5)	RSD of peak areas (n=5)
Reproducibility	single column	1.72%	7.78%
	different columns (n=3)	7.40%	6.28%
Life time	single column	11.67% (during 72h)	5.05%

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Figure 1. Scheme of H_1R-CMC–HPLC–MS method. Position A: Samples were injected into H_1R-CMC model (first-dimensional). Retained fraction on H_1R-CMC model was enriched onto a ODS enrichment column (EC1). Position B: after switching of the two-position ten-port valve, retained fraction was eluted from EC1 into the second-dimensional HPLC–MS system for chromatographic separation and MS identification. CMC, H_1R-CMC column; ODS, Shim-pack octadecylsilyl column; EC, enrichment column; UV, UV detector; DAD, diode array detector; MS, LC/MS-2010EV mass spectrometer.

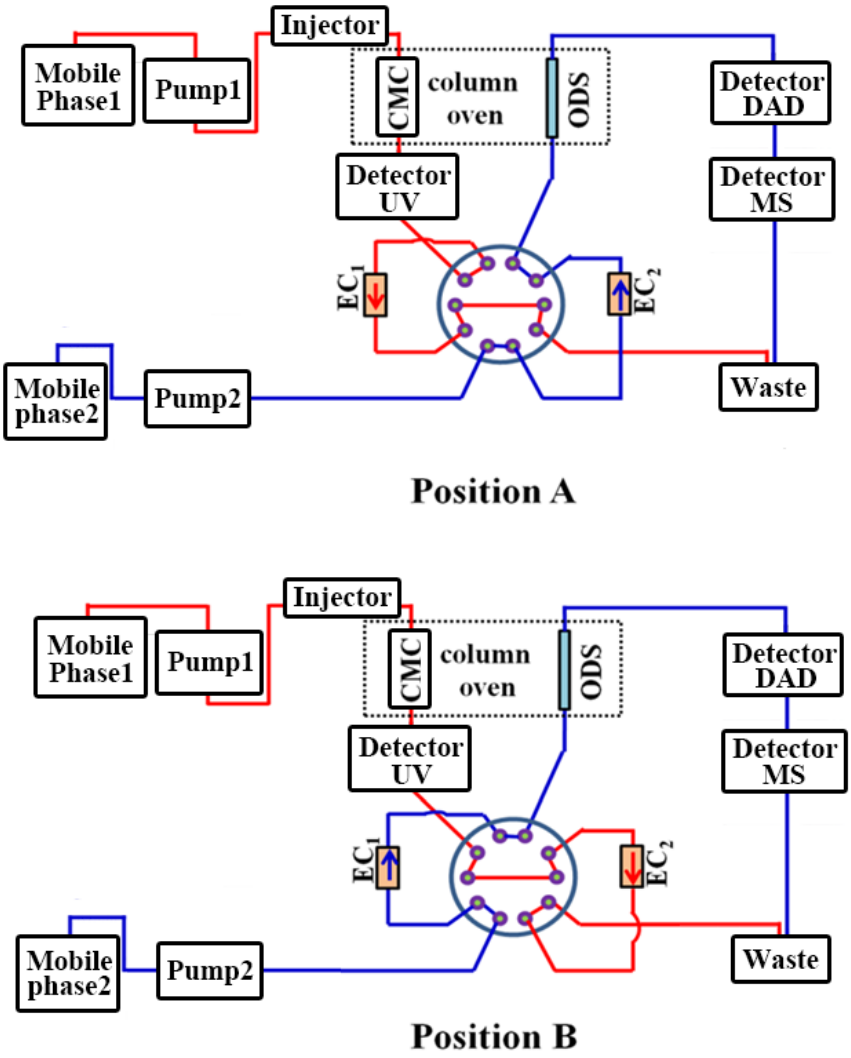

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Figure 2. Chromatograms of mixed standards solution analyzed by H_1R-CMC–HPLC–MS method. (A) The H_1R-CMC chromatogram of mixed standard solution, including retained fraction R1 and non-retained fraction R0 (between the two dotted lines). (B) The direct HPLC–MS chromatogram of the mixed standard solution. (C) HPLC–MS chromatogram of retained fraction R1, with a main retention peak identified as diphenhydramine hydrochloride (*m*/*z* 256). (D) HPLC–MS chromatogram of non-retained fraction R0. (a) tamsulosin, (b) diphenhydramine hydrochloride, (c) nitrendipine, and (d)gefitinib.

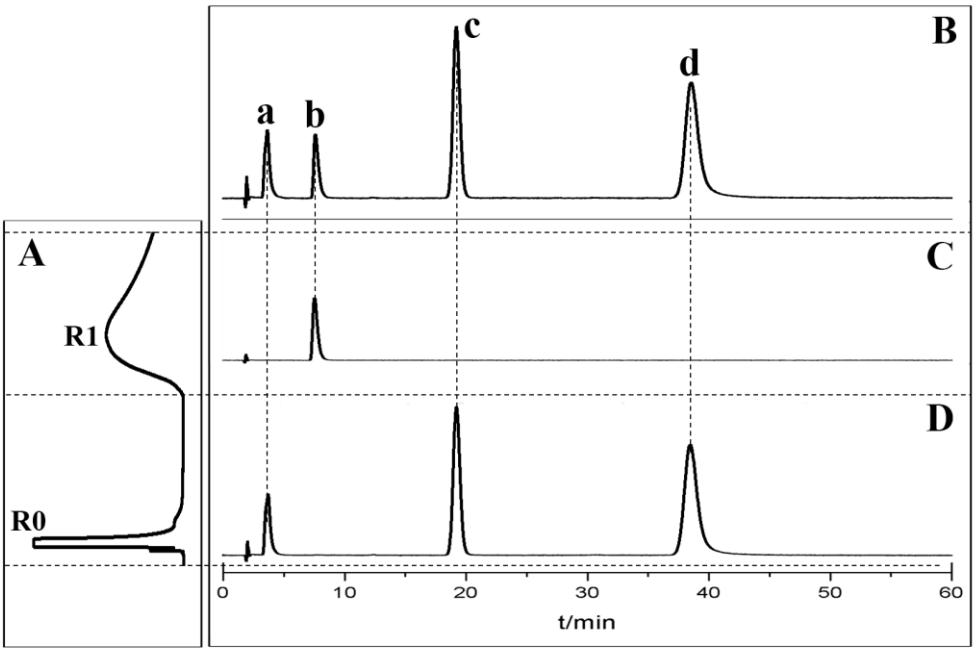

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Figure 3. Chromatograms of Yujin injection analyzed by H_1R-CMC–HPLC–MS method. (A) The H_1R-CMC chromatogram of Yujin injection, including one retained fraction R (between the two dotted lines). (B) The HPLC–MS chromatogram of retained fraction R, identified as sodium houttuyfonate (*m*/*z* 198). (C) The direct HPLC–MS analysis of the Yujin injection.

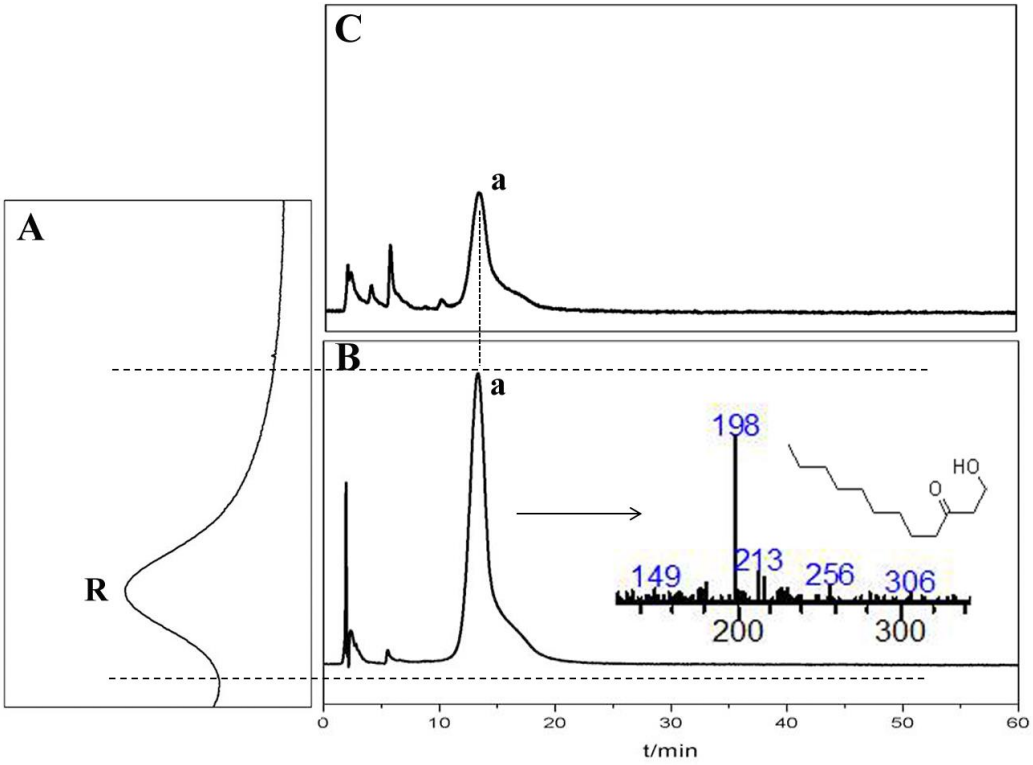

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Figure 4. Chromatograms of the standard solution of sodium houttuyfonate analyzed by H_1R-CMC–HPLC–MS method. (A) The H_1R-CMC chromatogram of sodium houttuyfonate standard solution (0.01 mg/mL), including retained fraction R1 (between the two dotted lines). (B) The HPLC–MS analysis of retained fraction R1, identified as sodium houttuyfonate (*m*/*z* 198). (C) The direct HPLC–MS analysis of the standard solution of sodium houttuyfonate.

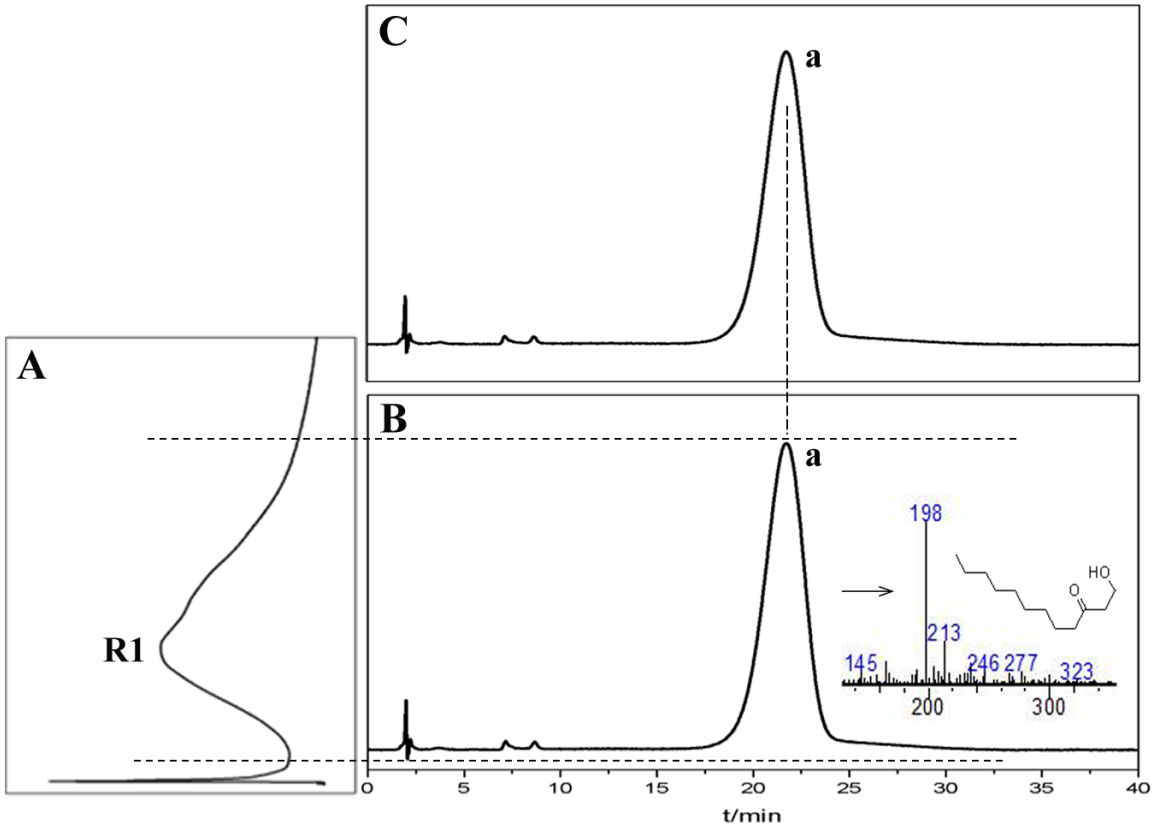

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Figure 5. The effects of Yujin injection and sodium houttuyfonate standard on cytosolic free Ca^{2+} concentration of H_1R-HEK293 cells, respectively. (A) Effects of Yujin injection on cytosolic free [Ca^{2+}]. (B) Effects of standard solution of sodium houttuyfonate on cytosolic free [Ca^{2+}].

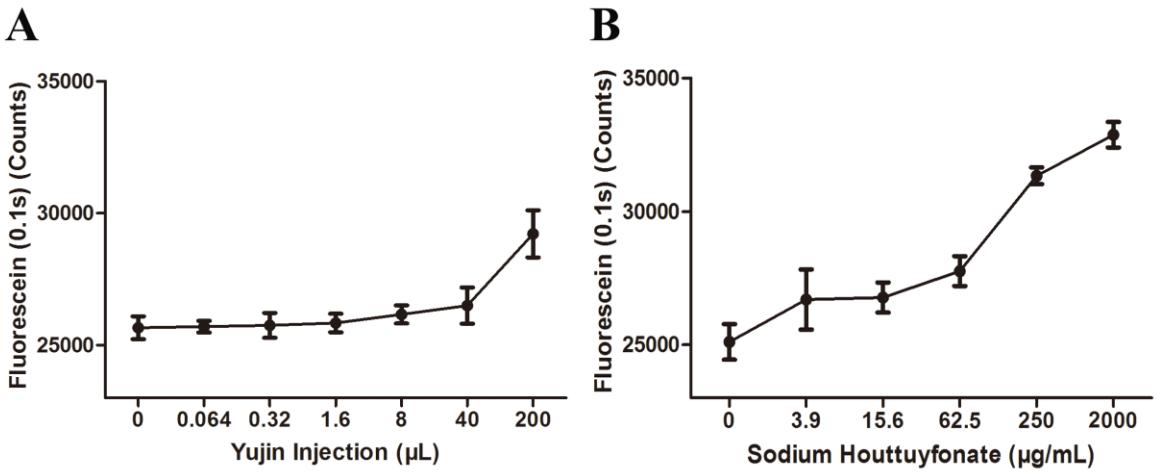

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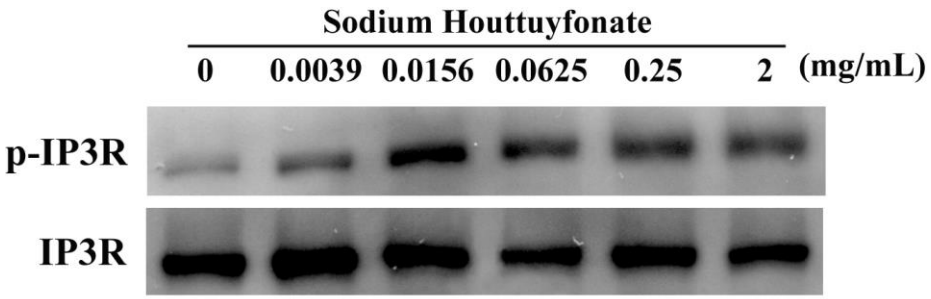
Figure 6. Effect of sodium houttuyfonate on protein expression of IP3R in H_1R-HEK293 cells. Cells were treated with different concentrations of sodium houttuyfonate for 5 min before extracting proteins with lysis buffer.



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