

## **Simultaneous identification of the three active constituents in lung-ventilating-regulating oral liquid by RP-HPLC.**

**Lina Lu<sup>1,2\*</sup>, Yanhua Zuo<sup>3</sup>, Yaoxia Wang<sup>3</sup>, Yongqing Shi<sup>1</sup>, Zuochong Liang<sup>1</sup>**

<sup>1</sup>Chemical Engineering Institute, Northwest University for Nationalities, Lanzhou, PR China

<sup>2</sup>Key Laboratory for Utility of Environment-Friendly Composite Material and Biomass, Universities of Gansu Province, Lanzhou, PR China

<sup>3</sup>Department of Pharmacy, the Affiliated Hospital of Qingdao University, Qingdao, PR China

### **Abstract**

**Problem statement:** Lung-Ventilating-Regulating Oral Liquid is specified for cough, antipathy to cold by fever, nasal obstruction or discharge, headache, anhidrosis, and pain. It has effects of releasing exterior syndrome, dissolving cold, smoothing lung, and get rid of cough.

**Objective:** To develop a High Performance Liquid Chromatography (HPLC) method for ephedrine, hesperidin, and baicalin in Lung-Ventilating-Regulating Oral Liquid.

**Methods:** The three active constituents were identified in an Agilent TC-C18 (2) chromatographic column (250 mm × 4.6 mm, 5 μm), with 0.2% phosphoric acid solution-methyl cyanides as mobile phase, which was performed at a gradient elution column temperature of 25°C, and a flow rate of 0.8 ml·min<sup>-1</sup>. Then the eluate was detected at detection wavelengths of 207 nm (for ephedrine) and 278 nm (for hesperidin and baicalin).

**Results:** Under the chromatographic conditions, ephedrine, hesperidin, and baicalin were well separated, which showed good linear relationships at 0.158-2.370, 0.164-4.100, and 0.160-4.000 μg, respectively. And coefficients of recovery of these three kinds of samples showed 100.2%, 98.7%, and 97.8%, respectively.

**Conclusion:** The developed method is convenient, accurate, and well repeatable, and consequently can be applied for the quality control of Lung-Ventilating-Regulating Oral Liquid.

**Keywords:** Baicalin, Ephedrine, Hesperidin, High performance liquid chromatography (HPLC), Chromatographic conditions, Phosphoric acid solution, Lung-ventilating-regulating oral liquid.

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### **Introduction**

Lung-Ventilating-Regulating Oral Liquid is an oral fluid, which is extracted and purified mainly from eleven kinds of Chinese medicinal materials [1,2]. It has effects of relieving exterior syndrome, dissipating cold, facilitating lung, and relieving cough. It is indicated for cough, aversion to cold with fever, nasal obstruction or discharge, headache, anhidrosis, and ache [3]. The formula includes cultivated purple perilla leaf (144 g), hogfennel root (96 g), balloonflower root (96 g), bitter apricot seed (fried) (72 g), Chinese ephedra herb (96 g), pinelliae tuber (fried with honey) (72 g), hoelen (96 g), bitter orange (96 g), baikal skullcap root (96 g), seasoned orange peel (96 g), and licorice root (72 g). The quality standard includes identification of ephedrine by HPLC, identification of seasoned orange peel and ephedrine by TLC, and inspections of liquid description, relative density, and pH value [4]. In the formula, ephedrine, the main extract of Chinese ephedra herb, has effects of relaxing bronchial smooth muscle and contracting

blood vessel, as well as marked central excitation effect [5]; hesperidin, the main extract of seasoned orange peel, has effects of improving deficiency of Qi and blood and lymphatic system hypofunction, expelling dampness, and eliminating sputum [6]; and baicalin, the main extract of baikal skullcap root, has effects of inhibiting bacterial growth, promoting urination, eliminating inflammation, anti-anaphylaxis, and relieving muscular spasm [7]. These effects are accordant with the functions and indications of the preparation. Currently, there are only reports on identification of ephedrine [8-10], hesperidin and baicalin [11] in Lung-Ventilating-Regulating Pills or Oral Liquid yet. While in this study, the amount of ephedrine, hesperidin, and baicalin in Lung-Ventilating-Regulating Oral Liquid was simultaneously determined by HPLC gradient elution [12,13], which provided reference foundations for better quality control of the preparation.