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Fast high-performance liquid chromatography analysis of phenethylamine alkaloids in *Citrus* natural products on a pentafluorophenylpropyl stationary phase

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Abstract

In this study, the chromatographic performance of a pentafluorophenylpropyl (PFPP) stationary phase was evaluated for the rapid separation of phenethylamine alkaloids (i.e. (±)-octopamine, (±)-synephrine, tyramine, *N*-methyltyramine and hordenine) in *Citrus aurantium* plant material (fruits and peel), various *Citrus* species, extracts and dietary supplements claiming to contain *C. aurantium*. The problems of phenethylamine alkaloid separation, such as peak tailing, low retention and low resolution, were successfully solved with this stationary phase. The parameters used for the method optimization included the mobile phase counter ion concentration and column temperature. A Discovery HS F5 column (150 mm × 4.6 mm i.d., 5 μm) was used, with an isocratic mobile phase composed of 10 mM ammonium acetate in 90:10 ACN–H_2O (v/v), at a flow rate of 1.0 mL/min. The column temperature was set at 20 °C. The photodiode array detector monitored the eluent at 225 nm. The total analysis time was 10 min. The validation parameters, such as linearity, sensitivity, accuracy, precision and specificity, were found to be highly satisfactory. With a simple sample preparation procedure, different matrices were successfully analyzed for their alkaloid content. The results indicated that the products on sale, labeled as dietary supplements, vary widely in the quantitative composition of the active constituents: the amount of (±)-synephrine, the major alkaloid, in such products ranged from 0.65 to 27.41 mg/g. The other compounds were either not detected or were present at low levels. The developed method can be considered suitable for the quality control of *Citrus* plant material and commercial products.

Keywords: *Citrus aurantium*; Phenethylamine alkaloids; (±)-Octopamine; (±)-Synephrine; Tyramine; *N*-Methyltyramine; Hordenine; Pentafluorophenylpropyl stationary phase; HPLC

1. Introduction

Citrus aurantium (bitter orange) is a plant belonging to the Rutaceae family, the fruit extracts of which have recently been used for the treatment of obesity in humans. The main compounds responsible for this activity are the phenethylamine alkaloids, mainly (±)-octopamine, (±)-synephrine, tyramine, *N*-methyltyramine and hordenine (Fig. 1) [1–3].

Synephrine has effects on human metabolism that could help to reduce fat mass in obese people, because it stimulates lipolysis, increases the metabolic rate and promotes the oxidation of fat through increased thermogenesis [4,5]. However, it is known that synephrine and the other alkaloids found in *C. aurantium* affect the cardiovascular system by adrenergic stimulation [6–8].

Synephrine is an alkaloid that is similar in structure to ephedrine, the main active component of plants of the genus *Ephedra*. After the ban of *Ephedra*-containing dietary supplements in April 2004 [9,10], "*Ephedra*-free" dietary supplements for weight loss were introduced [11,12]. *C. aurantium* is an ingredient in many of these *Ephedra*-free dietary supplements, which often contain concentrates of other herbs that are rich in caffeine. The combination of synephrine and caffeine has the same potential to induce arrhythmia, hypertension, heart attacks and strokes as the combination of ephedrine and caffeine [7]. Furthermore, *C. aurantium* extracts inhibit intestinal cytochrome P450 (CYP) 3A4, which is an enzyme responsible

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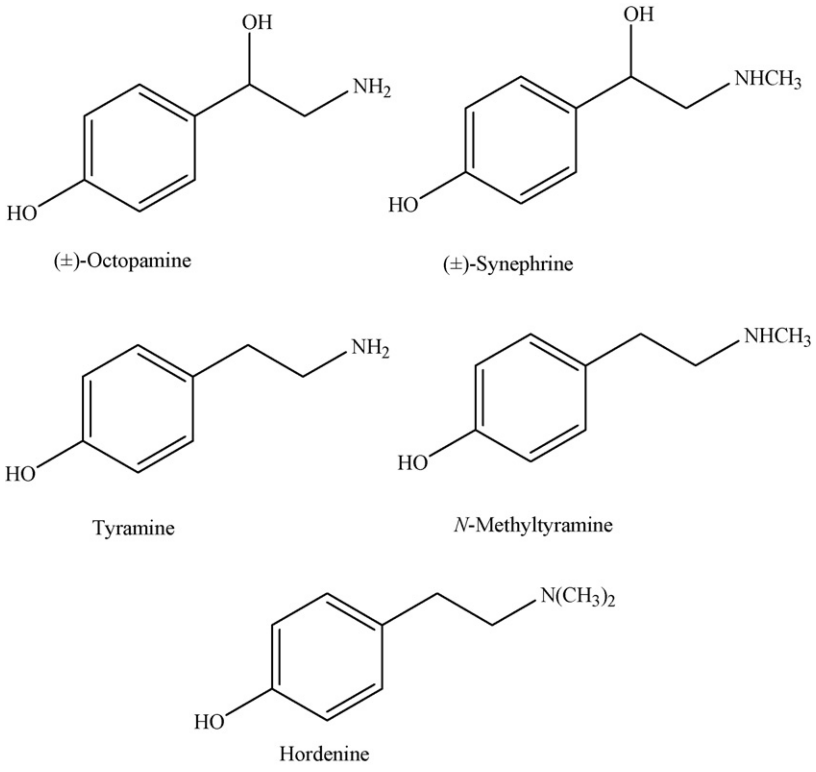

Fig. 1. Chemical structures of phenethylamine alkaloids isolated from *Citrus*.

for the metabolism of a large number of drugs. Thus, they can cause an alarming increase in the plasma levels of many drugs [13,14].

A variety of chromatographic and electrophoretic separation techniques have been used for the qualitative and quantitative determination of phenethylamine alkaloids in *C. aurantium* and related species [15]. Several analytical techniques involving high-performance liquid chromatography (HPLC) [13,16–33], gas chromatography (GC) [10] and capillary electrophoresis (CE) [34–36] have been reported for determination of these alkaloids [15]. HPLC with UV detection is the most frequently used analytical technique for detection of *Citrus* alkaloids, and most separations are done on reversed-phase columns; in several methods, the mobile phase contains an ion-pair reagent to increase the peak symmetry and resolution of the target analytes [13,16–18]. Other studies have used RP-HPLC with UV detection without an ion-pair reagent [19–21]. Although UV detection is one of the most frequently used detection methods for phenethylamine alkaloids, electrochemical detection [22] and mass-spectrometry (MS) detection [23,24] have been applied for improved specificity and sensitivity. HPLC has also been used for the enantioselective analysis of synephrine in *C. aurantium* natural products [25–27]. Furthermore, considering that various compounds that are likely to increase the risk of secondary effects and constitute a serious health concern are common in dietary supplements containing synephrine, several HPLC methods described in the literature allow the simultaneous analysis of *Citrus* and *Ephedra* alkaloids [28–33]. The majority of the above described RP-HPLC methods use columns packed with C_{18} porous spherical silica particles. However, it is well known that these amine compounds can strongly interact with the free silanols on the surface of the stationary phase, which directly causes the peak tailing of the analytes and affects the resolution and the quantitative analyses. Therefore, sodium dodecyl sulphate (SDS) and related compounds have been widely used as ion-pair reagents to suppress peak tailing and increase the retention of the phenethylamine alkaloids. The main limitation of ion-pair reagents is that their poor volatility and ion-suppressing effects make methods using them less amenable to MS analysis [15].

As weight reduction is a profitable healthcare market segment, a fully validated method for a simple, fast and reliable analysis of all the phenethylamine alkaloids in *C. aurantium* samples is essential to ensure the quality and safety of commercial products. The development and validation of an HPLC method fulfilling these requirements was the aim of the present study. An earlier work had demonstrated that a pentafluorophenylpropyl (PFPP) stationary phase showed excellent separation capacity of ephedrine alkaloids, the active constituents of *Ephedra* species, with high retention and resolution [31]. However, this method had not been validated and its use on complex matrices had not been reported. In this study, an HPLC method on a PFPP stationary phase was optimized and fully validated, for the first time, for the separation and quantitative determination of phenethylamine alkaloids ((±)-octopamine, (±)-synephrine, tyramine, *N*-methyltyramine and hordenine) in *C. aurantium* natural products and related species. The choice of this stationary phase markedly improved the retention and resolution of these difficult analytes. For controlling the retention, several influencing factors, such as the mobile phase