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Strategies for supercritical fluid extraction of hyoscyamine and scopolamine salts using basified modifiers

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Abstract

The supercritical fluid extraction behaviors of hyoscyamine and scopolamine were investigated and found to be highly dependent upon the chemical nature of the compounds. Free bases of hyoscyamine and scopolamine were freely soluble in supercritical CO_2 with increasing temperature and pressure; however, the salts of these alkaloids were not soluble under any experimental conditions. It was found that alkaline modifiers such as methanol basified with diethylamine could enhance the solubilities and extraction yields of these alkaloids from plant matrices as compared to other modifiers.

Keywords: Extraction methods; *Scopolia japonica*; Hyoscyamine; Scopolamine; Tropane alkaloids; Alkaloids

1. Introduction

In view of increasing environmental concerns about the use of organic solvents in the extraction of natural products, there has been a growing interest in supercritical fluid extraction (SFE) as an alternative to conventional organic solvent extraction methods [1–4]. Among the various groups of plant natural products, alkaloids have been used as target compounds for SFE more than any other secondary metabolites because of their diverse biological activities [5–11].

To date, CO_2 among the useful supercritical fluids has been most widely employed for the extraction of alkaloids due to its low critical point, low toxicity and chemical inertness [12]. However, most alkaloids are too polar to be sufficiently extracted by pure CO_2. Accordingly, a polar solvent such as methanol or water has been required as a modifier in SFE of alkaloids [8–11]. In addition, other supercritical fluids such as N_2O [13,14] and CHF_3 [15] have been utilized to enhance SFE efficiencies of alkaloids. However, several problems including the limitation to the percentage of modifiers and the environmental hazards of the alternative supercritical solvents have led to the development of another SFE method before it became universally accepted as an extraction method for alkaloids.

During the present study on the application of SFE to alkaloids, it was planned to extract hyoscyamine and scopolamine (Fig. 1), which are important tropane alkaloids as parasympatholytic [16], from a plant of origin. In preliminary experiments, it was found that free bases of hyoscyamine and

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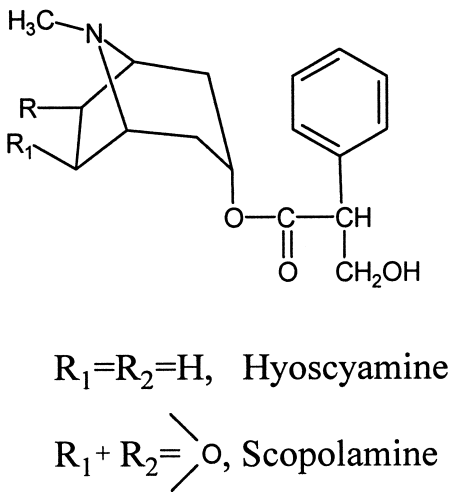


Fig. 1. Chemical structures of hyoscyamine and scopolamine.

scopolamine were quite soluble in pure supercritical CO_2, even under mild supercritical conditions (40°C and 10.2 MPa), but these compounds could not be extracted at all from plant materials (*Scopolia japonica* roots and aerial parts). This discrepancy was assumed to be due to the fact that hyoscyamine and scopolamine exist in the form of salts in the plant [17]. For this reason, these tropane alkaloids have been extracted from plant materials traditionally by an extraction process using basic solutions such as ammonia or calcium hydroxide [18–21]. Accordingly, the use of basified modifiers in SFE, rather than simple polar modifiers were investigated with the hope that the extraction efficiencies of hyoscyamine and scopolamine from their plant of origin would be enhanced.

2. Experimental

2.1. Chemicals and reagents

Hyoscyamine hydrochloride, scopolamine hydrochloride, hyoscyamine free base, palmitic acid methyl ester and acetaminophen were purchased from Sigma (St. Louis, MO, USA). Scopolamine free base was prepared from scopolamine hydrochloride. HPLC-grade methanol, chloroform, ethyl acetate and deionized water were purchased from J.T. Baker (Phillipsburg, NJ, USA). Diethylamine (99%) and NH_4OH (28%) were obtained from Duksan (Yongin, Kyungki Province, South Korea). A silylation reagent, *N,O-bis*(trimethylsilyl)acetamide (BSA) in dimethylformamide (DMF), was purchased from Pierce (Rockford, IL, USA).

2.2. Plant materials

Scopolia japonica Maxim. was collected at Samhak Mountain, Chunchun, Kangwon Province, South Korea in April 1995. The authenticity was confirmed by one of the authors (J.K.). A voucher specimen (SNUPH-0075) has been deposited at the Herbarium of Medicinal Plant Garden, College of Pharmacy, Seoul National University. The plant materials were divided into aerial parts and roots, and were dried at 40°C for three days in a vacuum oven. They were pulverized and sieved (the particle size was under 0.71 mm).

2.3. Organic solvent extraction of plant materials

The dried and pulverized plant materials (500 mg) were extracted with 50 ml of a mixture of chloroform–methanol–28% NH_4OH (15:5:1) under reflux for 3 h. The extract was suspended with 20 ml of water and partitioned with chloroform (3×20 ml). The chloroform portion was evaporated to dryness [22].

2.4. Supercritical fluid extraction for pure compounds

SFE was performed on an Isco supercritical fluid extractor, Model SFX 3560 equipped with two Isco 260 D syringe pumps (Lincoln, NE, USA) using CO_2 (99.9%, Seoul Gas, Seoul, South Korea) in the pressure range of 10.2–34.0 MPa at 40 and 60°C. In each experiment, 200 mg each of hyoscyamine free base, scopolamine free base, hyoscyamine hydrochloride and scopolamine hydrochloride were loaded into an extraction cell (57 mm×20 mm I.D., Isco). The temperature of the restrictor was kept at 80°C (±2°C) and the static extraction time was 45 min. The flow-rate was 0.8–1.2 ml/min through the extraction vessel during the dynamic extraction time. The total amount of consumed CO_2 was 10 ml for

the free bases of hyoscyamine and scopolamine, and 30 ml for salts of these compounds during the dynamic extraction under each condition. For the evaluation of the effects of the modifiers such as methanol, water, 10% (v/v) diethylamine in methanol, and 10% (v/v) diethylamine in water on the extractabilities of salts, each modifier was continuously incorporated into the extraction cell at the concentrations of 1, 5 and 10% (v/v) through a syringe pump at 60°C and 34.0 MPa. All extracted analytes were collected in 20-ml vials containing 10 ml of methanol.

2.5. Supercritical fluid extraction of plant materials

SFE of the plant materials was performed in the same apparatus used in the experiments on SFE of pure compounds. Pure CO_2 or CO_2 modified with methanol, water, 10% (v/v) diethylamine in methanol, and 10% (v/v) diethylamine in water at the concentrations of 1, 5 10% (v/v), respectively, were used for the extraction at 60°C and 34.0 MPa. In each experiment, 250 mg of plant material was loaded in an extraction cell and the remaining volume was filled with glass wool. The static time was 15 min. All the experimental conditions except static time (15 min) were identical to those for SFE of pure compounds. The total amount of consumed CO_2 was 150 ml. In each extraction step, the extract was fractionated every 15 ml.

2.6. Gas chromatography–flame ionization detection (GC–FID) analysis

For the measurement of pure compound extractabilities of hyoscyamine and scopolamine free bases, the methanol extracts were evaporated under reduced pressure, and dissolved in an appropriate amount of chloroform (1–10 ml). Then, 1 ml of the chloroform solution was re-evaporated together under a N_2 stream with 1 mg of palmitic acid methyl ester solution as an internal standard, and dissolved in 1 ml of chloroform for GC analysis. SFE extracts of hyoscyamine hydrochloride, scopolamine hydrochloride, the plant materials and the dried organic solvent extracts were dissolved in 2 ml methanol and transferred into a reaction vial. Acetaminophen (100 μg) was used as an internal standard and added to each solution and evaporated under a N_2 stream. Acetaminophen was used as an internal standard for the extracts of salts and plant materials due to insolubility of these compounds in nonpolar solvent such as chloroform, so they should be trimethylsilyl (TMS) derivatized for enhancing volatility. The extracts of the salts and plant materials were derivatized with 100 μl of BSA in DMF at 75°C for 45 min. GC–FID was performed on a Hewlett-Packard (Avondale, PA, USA) 5890 series II gas chromatograph equipped with a HP 3395 integrator and a capillary GC column (Ultra 1, crosslinked methyl siloxane, 25 m×0.32 mm, film thickness 0.52 μm, HP). Helium was used as a carrier gas at a rate of 3.7 ml/min. The split ratio was 20:1. The oven temperature was increased from 150 (1 min hold) to 280°C at a rate of 8°C/min (20 min hold). The injector and detector temperatures were 200°C and 280°C, respectively. Quantitative standard curves (five points covering the range of values found in the sample extracts) for free bases of hyoscyamine and scopolamine and TMS ethers of these alkaloidal salts were generated using each internal standard.

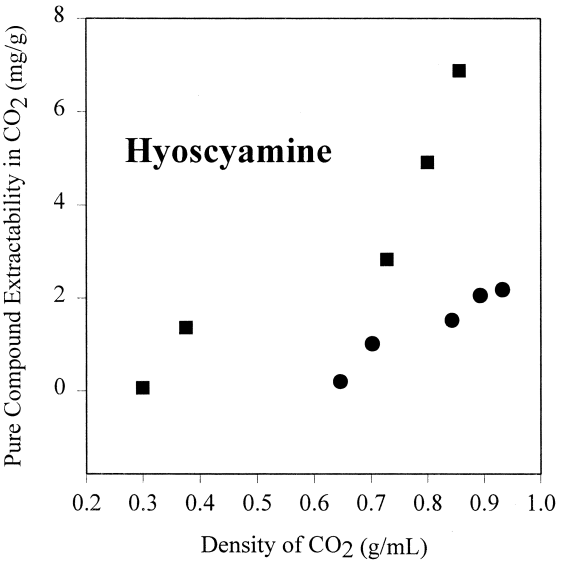
3. Results and discussion

3.1. Pure compound extractabilities of the free bases and salts of hyoscyamine and scopolamine in supercritical CO_2

Hyoscyamine and scopolamine were not extracted at all from the roots and the aerial parts of *S. japonica* by pure supercritical CO_2 in the preliminary tests, because most alkaloids are often stored as salt forms in vacuoles of plant cells [17,23]. Therefore, the solubilities of these salts must be compared with those of free bases prior to conducting SFE. In the present investigation, the effects of static time and flow-rate for the equilibrium state between the solute and solvent were not considered, since the purpose of this work was to measure only the effects of various modifiers on the extractabilities of the target compounds. Thus, the term ''pure compound extractability'' was used instead of ''solubility'' in

the strict sense. Their extractabilities (mg/ml) increased with increasing pressure in a similar manner to other compounds reported by previous researchers [24]. The extractabilities of hyoscyamine and scopolamine free bases per consumed weight of CO_2 (mg/g) are plotted as a function of the density of CO_2 in Fig. 2.

Although there were some differences on the effects of temperature and pressure between two compounds, the free bases of hyoscyamine and scopolamine were found to be highly soluble in supercritical CO_2 as determined with 5.89 and 11.29 mg/ml, respectively, at 60°C and 34.0 MPa. However, the hydrochloride salts of these compounds were scarcely extracted by pure CO_2 at any condition employed as same as the results of plant materials. Therefore, it was necessary to develop a procedure to enhance the extractabilities of hyoscyamine and scopolamine salts in CO_2.



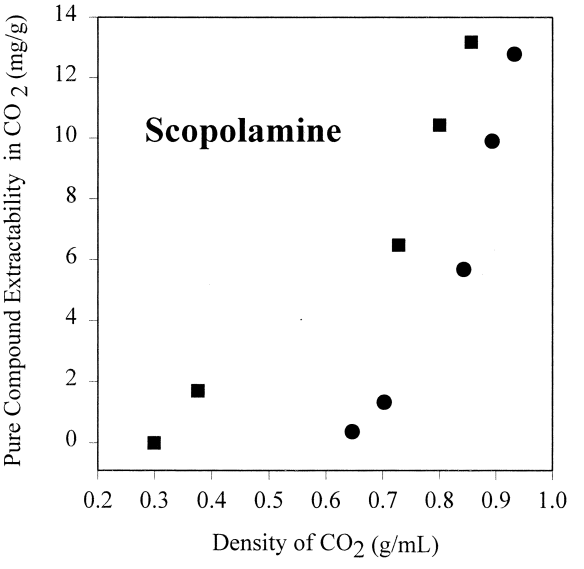


Fig. 2. Effect of density of CO_2 (g/ml) on the pure compound extractabilities of hyoscyamine and scopolamine free bases at 40°C (●) and 60°C (■). SFE conditions: 45 min static, 1.0 ml/min flow-rate, 10 ml of CO_2 was eluted under each condition during dynamic extraction.

3.2. Effect of modifiers on the extractabilities of hyoscyamine and scopolamine hydrochloride salts

To improve supercritical CO_2 extractabilities of hyoscyamine and scopolamine hydrochloride salts, an appropriate modifier raising the polarity of CO_2 had to be used. The effect of methanol and water is shown in Fig. 3. It was found that addition of methanol drastically increased the extraction yield of hyoscyamine and scopolamine. However, water did not show any significant influence on the extractabilities of hyoscyamine, although it slightly increased in the case of scopolamine. The inefficiency of water relative to methanol may be due to the fact that water could not sufficiently improve the polarity of CO_2 as much as methanol, since only 0.5% (v/v) of water can be completely miscible with CO_2 [25].

In the next part of the investigation, diethylamine as a basifying agent was added to methanol and water to improve the extractabilities of hyoscyamine and scopolamine hydrochloride. The effects of basified modifiers on the extractabilities are shown in Figs. 4 and 5. The addition of diethylamine (10%) to methanol or water dramatically enhanced the extractabilities of hyoscyamine and scopolamine hydrochloride when compared to pure methanol or water. This may be due to the fact that the salts are changed to free bases by minor addition of methanol or water basified with diethylamine, so supercritical CO_2 could freely dissolve the free bases. When the

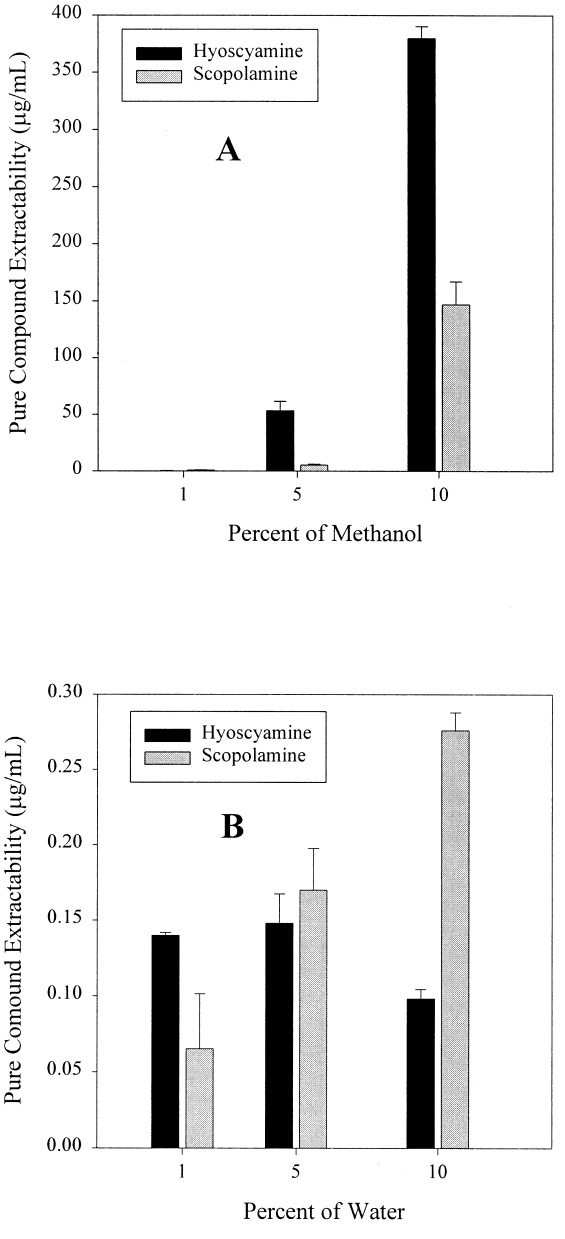


Fig. 3. Effect of methanol (A) and water (B) on the pure compound extractabilities (mg/l) of hyoscyamine and scopolamine hydrochloride. SFE conditions: 60°C, 34.0 MPa, 45 min static, 1.0 ml/min flow-rate, 30 ml of CO_2 was eluted under each condition during dynamic extraction.

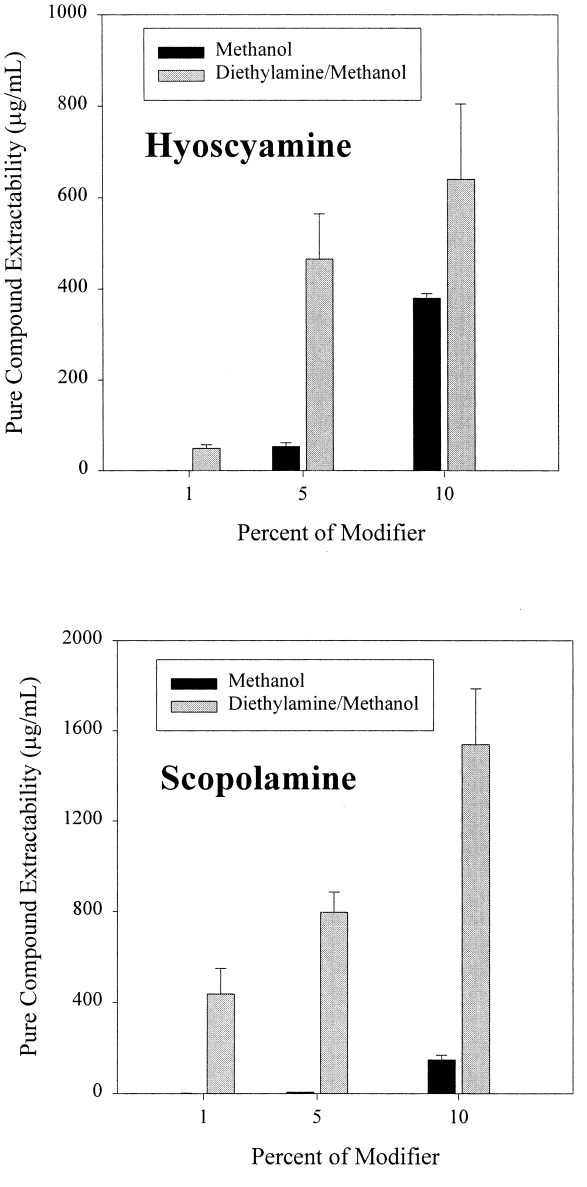


Fig. 4. Comparison of the extractabilities of hyoscyamine and scopolamine hydrochloride using methanol basified with diethylamine (10%, v/v) and pure methanol, respectively. SFE conditions: 60°C, 34.0 MPa, 45 min static, 1.0 ml/min flow-rate, 30 ml of CO_2 was eluted under each condition during dynamic extraction.

extractabilities of diethylamine–methanol as a modifier were compared with those of diethylamine–water, the former was found to be more effective on

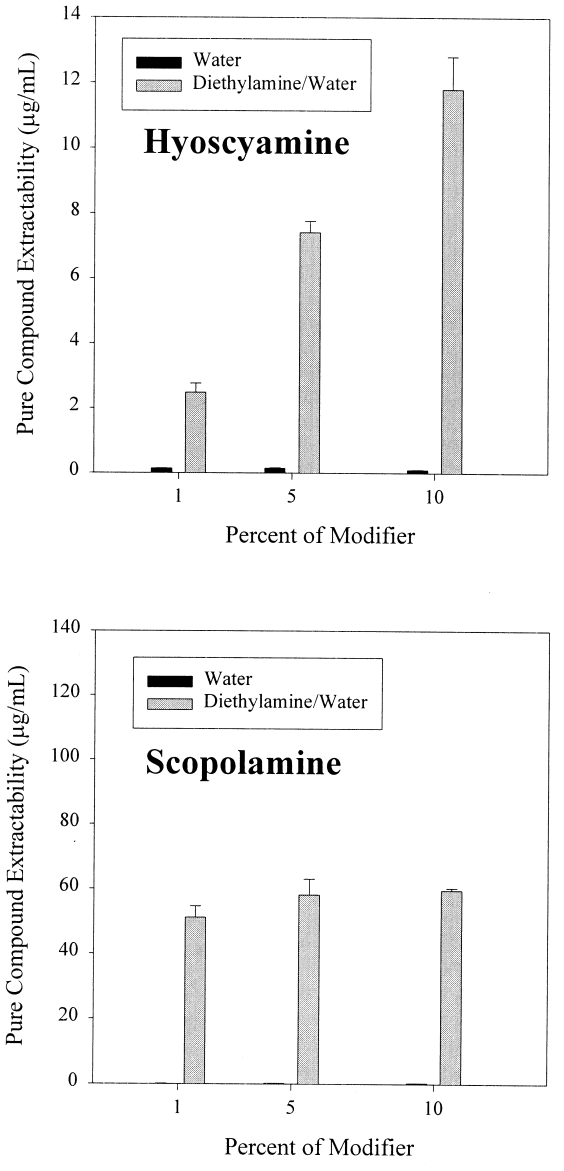


Fig. 5. Comparison of the extractabilities of hyoscyamine and scopolamine hydrochloride using water basified with diethylamine (10%, v/v) and pure water, respectively. SFE conditions: 60°C, 34.0 MPa, 45 min static, 1.0 ml/min flow-rate, 30 ml of CO_2 was eluted under each condition during dynamic extraction.

the extractabilities of hyoscyamine and scopolamine salts than the latter.

On the basis of these results on the pure compound extractabilities, SFE was applied to the extraction of hyoscyamine and scopolamine from plant matrices.

3.3. Extraction efficiencies of hyoscyamine and scopolamine from roots and aerial parts of S. japonica using a variety of modifiers

The yields of hyoscyamine and scopolamine from the roots and aerial parts by SFE are listed in Tables 1 and 2. These values from both plant parts were greatly enhanced by the addition of diethylamine to methanol or water. From the results of pure compound extractability, methanol and diethylamine in methanol (10%, v/v) were much better for both compounds than water and diethylamine in water (10%, v/v). The trends of the extractabilities of hyoscyamine obtained from plant materials were in good agreement with those of pure compound extractabilities. However, in the case of scopolamine, there was not a great difference between the results using methanol and water based modifiers. Morrison et al. reported that basified water was more effective in SFE of cocaine, another tropane alkaloid, from several matrices than basified methanol and they suggested that it was not limited by analyte solubility, but rather, by the desorption of cocaine from the matrices [26]. Thus, in the case of scopolamine, it was also suggested that the main factor of the modifiers in SFE is not the solubility, but the interaction with the plant matrix.

The cumulative recoveries of hyoscyamine and scopolamine versus the consumed CO_2 are shown in Fig. 6. The variation of static time in the range of 15–60 min showed little influence on the SFE behavior. According to the study of SFE for natural products, the recovery of each target compound reached at plateau within 10 min [27,28]. However, the recoveries of hyoscyamine and scopolamine reached only about 50% at 60 min (60 ml at a flow-rate of 1.0 ml/min). This slow extraction profile might be due to the fact that most alkaloids are located in the cell vacuole of plant tissue, so the supercritical fluid must penetrate the plant cell wall barriers for sufficient extraction, in a different manner from the components in cell wall or epicuticular

Table 1
Effect of different volumes of modifiers on the SFE yields (mg/g) of hyoscyamine and scopolamine from the roots of *S. japonica*

% (v/v) of the modifiers	SFE[a] (mg/g) (%, RSD)[b]							
	Methanol		Water		Diethylamine–methanol[c]		Diethylamine–waterd	
	Hyoscyamine	Scopolamine	Hyoscyamine	Scopolamine	Hyoscyamine	Scopolamine	Hysocyamine	Scopolamine
1	0.38 (10.8)	0.12 (21.5)	0.15 (38.1)	0.20 (12.1)	0.73 (6.6)	0.14 (3.1)	0.53 (14.8)	0.18 (14.0)
5	1.91 (3.3)	0.15 (7.8)	0.34 (2.3)	0.23 (3.8)	4.58 (8.3)	0.23 (10.7)	0.66 (9.0)	0.23 (9.3)
10	3.38 (6.6)	0.16 (8.1)	0.14 (17.2)	nd[e]	6.24 (2.2)	0.24 (6.4)	0.20 (13.6)	0.22 (13.4)
Organic solvent extraction[f]								
Hyoscyamine	6.35 (11.4)	Scopolamine	0.26 (10.2)					

[a] The temperature and pressure were 60°C and 34.0 MPa, respectively. The static time was 15 min. The total amount of consumed CO_2 was 150 ml during dynamic extraction.
[b] The values were based on triplicate analysis.
[c] 10% Diethylamine in methanol.
[d] 10% Diethylamine in water.
[e] Not detected.
[f] The extraction solvent was chloroform–methanol–28% NH_4OH (15:5:1).

wax layer, and also the low pH of vacuole could make alkaloids tightly bind to the matrix.

4. Conclusion

Though there was little difference in the enhancement of the extractabilities depending on the plant matrix, it is evident that the basified modifiers provided a greater improvement of the extractabilities of hyoscyamine and scopolamine when compared to pure methanol or water. These results demonstrate that supercritical CO_2 with basified modifiers can extract the salts of hyoscyamine and scopolamine from plant materials in the form of their free bases, which then become freely soluble in

Table 2
Effect of different volumes of modifiers on the SFE yields (mg/g) of hyoscyamine and scopolamine from the aerial parts of *S. japonica*

% (v/v) of the modifiers	SFE[a] (mg/g) (%, RSD)[b]							
	Methanol		Water		Diethylamine–methanol[c]		Diethylamine–water[d]	
	Hyoscyamine	Scopolamine	Hyoscyamine	Scopolamine	Hyoscyamine	Scopolamine	Hyoscyamine	Scopolamine
1	0.081 (35.6)	0.13 (27.1)	0.11 (8.3)	0.32 (12.1)	0.44 (14.2)	0.39 (32.9)	0.092 (11.4)	0.53 (12.1)
5	0.23 (25.0)	0.28 (37.6)	0.081 (6.2)	0.30 (11.3)	0.62 (5.4)	0.51 (8.1)	0.61 (27.8)	0.69 (26.8)
10	0.49 (15.5)	0.26 (19.8)	0.093 (7.7)	0.38 (7.4)	1.17 (7.4)	0.69 (3.2)	0.26 (15.1)	0.53 (4.3)
Organic solvent extraction[e]								
Hyoscyamine	1.56 (9.6)	Scopolamine	0.65 (2.3)					

[a] The temperature and pressure were 60°C and 34.0 MPa, respectively. The static time was 15 min. The total amount of consumed CO_2 was 150 ml.
[b] The values were based on triplicate analysis.
[c] 10% Diethylamine in methanol.
[d] 10% Diethylamine in water.
[e] The extraction solvent was chloroform–methanol–28% NH_4OH (15:5:1).

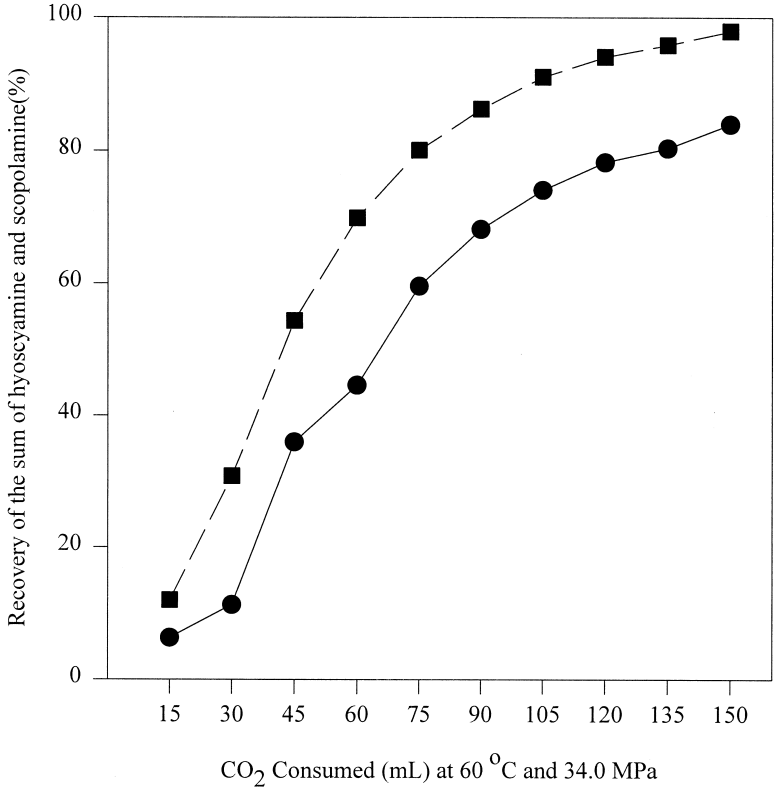


Fig. 6. Cumulative recoveries of the sum of hyoscyamine and scopolamine from roots (■) and aerial parts (●) of *S. japonica* using supercritical CO_2 modified with 10% (v/v) diethylamine in methanol at every 15 ml fraction. SFE conditions: 60°C, 34.0 MPa, 15 min static, 1.0 ml/min flow-rate.

nonpolar solvents. These results will prove useful for the SFE of other alkaloidal salts from other types of plant materials.

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