Korean J. Chem. Eng., **17**(6), 672-677 (2000)

Effects of Basic Modifiers on SFE Efficiencies of Ephedrine Derivatives from Plant Matrix

Jin Yeol Kim and Ki-Pung Yoo†

Department of Chemical Engineering, Sogang University, C.P.O. Box 1142, Seoul, Korea

(*Received 18 May 2000 • accepted 23 August 2000*)

Abstract—The effect of basified modifiers in supercritical CO_2 (SCF-CO_2) such as diethylamine has been investigated on the SFE efficiencies of ephedrine derivatives (e.g. methylephedrine, norephedrine, ephedrine, and pseudoephedrine) from aerial parts of *Ephedra sinica*. The addition of basified modifiers in SCF-CO_2 showed a greater enhancement of SFE efficiency of ephedrine derivatives than pure modifiers. These results might be due to the fact that the salts of *Ephedra* alkaloids (insoluble in CO_2) in plant tissues would be changed to their free bases (freely soluble in CO_2) by basified modifiers such as diethylamine in methanol. In addition to enhancing the solubilities, it could increase the degree of desorption of the compounds from plant matrix. Also, the SCF-CO_2 modified with methanol basified with diethylamine showed a difference of extractability of ephedrine and its diastereomer, pseudoephedrine.

Key words: Supercritical CO_2, Basified Modifier, Ephedrine Derivatives, *Ephedra sinica*

INTRODUCTION

Natural pharmaceutical products such as alkaloids have received a great deal of attention as the target compounds for supercritical fluid extraction (SFE) because of their diverse and intensive biological activities. A number of SFE applications on the extraction of alkaloids including caffeine, monocrotaline, senecionine, seneciphylline, thebaine, codeine, morphine, *O*-methylcariachine, protopine, α-allocryptopine, escolzine, californidine, sanguinorine, and chelerythine from plant materials have been reported by previous researchers [Elisabeth et al., 1991; Sugiyama et al., 1985; Ndiomu et al., 1988; Shaeffer et al., 1989; Bicchi et al., 1991; Janicot et al., 1990; Bugati et al., 1993]. However, in the extraction of alkaloids by using SCF-CO_2, there are some points to be considered. Carbon dioxide is so nonpolar that it is sparingly solubilize polar alkaloids. Moreover, most alkaloids do not exist as free bases (freely soluble in nonpolar solvent) but salt forms (insoluble in nonpolar solvents) conjugated with several acids in plant tissues due to their characteristic basicities [Tyler et al., 1981]. We intend to extract ephedrine derivatives, benzylamine alkaloids, from aerial parts of *Ephedra sinica* using SCF-CO_2. The aerial parts of *Ephedra sinica* have long been used in traditional medicine as a diaphoretic, anti-asthmatic and diuretic as well as for the treatment of bronchitis and acute nephritic oedema [Tang and Eisenbrand, 1992]. The alkaloids isolated from *E. sinica*, *Ephedra* alkaloids such as methylephedrine (**1**), norephedrine (**2**), ephedrine (**3**), pseudoephedrine (**4**) (Fig. 1) have been used for asthma, influenza and some types of inflammation [Tang and Eisenbrand, 1992].

In order to enhance the SFE efficiency of ephedrine derivatives, we performed SFE experiments including measurements of the effects of modifiers on the solubilities (pure compound extractabilities or pseudosolubilities) of ephedrine derivatives and desorption of them from a matrix. Based upon the results of solubilities and desorption from a matrix, SFE was performed on the extraction of the alkaloids from plant material (*E. sinica*).

$R_1=R_2= CH_3$; Methylephedrine (1)
$R_1=R_2= H$; Norephedrine (2)
$R_1=CH_3$, $R_2= H$; Ephedrine (3)

Pseudoephedrine (4)

Fig. 1. Chemical structures of methylephedrine (1), norephedrine (2), ephedrine (3), and pseudoephedrine (4).

EXPERIMENTAL

1. Plant Material

The aerial parts of *E. sinica* were obtained from Korea Export and Import Federation of Drugs, Seoul, Korea. The plant materials were dried at 40 °C for 24 hrs and pulverized.

2. Chemicals and Standards

The HPLC grade methanol and water were purchased from J. T. Baker Inc. (Phillipsburg, NJ. USA). Diethylamine (99%) and diethylether (95%) were obtained from Duksan Chemical Co. (Yongin, Kyungki-Do, Korea). N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), orcinol (98%), and pseudoephedrine free base (99%) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Hydrochloride salts of methylephedrine (>99%), norephedrine (>99%), ephedrine (>99%), and pseudoephedrine (>99%) were generously donated by B.-J. Cha (Dong-A Pharm. Co. Ltd., Anyang, Kyungki-Do, Korea).

3. Spiking Ephedrine Derivatives into Filter Paper

---

†To whom correspondence should be addressed.
E-mail: kpyoo@ccs.sogang.ac.kr

This paper was presented at The 5th International Symposium on Separation Technology-Korea and Japan held at Seoul between August 19 and 21, 1999.

Table 1. SFE experimental condition for each sample

Sample	Temperature (°C)	Pressure (MPa)	Flow rate (mL/min)	Static time (min)	Volume consumed (mL)
Pseudoephedrine free base	40 and 60	10.2-34.0	1.0	15	10
Salts of ephedrine derivatives	80	34.0	1.0	15	10
Spiked filter papers	80	34.0	1.0	15	50
Plant materials	80 and 100	34.0	1.0	15	50 and 100

1 g of filter paper disks (Advantec No. 2, Toyo Roshi Kaisha, Japan) were cut into *ca.* 0.7-1.2 cm diameter and placed in the extraction vessel. Each standard of ephedrine derivatives (0.2 mg) was spiked into the filter paper disks. Then the spiked samples were dried in vacuum oven at 40 °C for 24 hours.

4. Organic Solvent Extraction

The dried and pulverized plant materials (1.0 g) were extracted with 70 mL of 0.5 M H_2SO_4 for 12 hours [Zhang et al., 1988]. The filtered extract was adjusted to pH 11-13 by the addition of 6 M NaOH. 16 g of NaCl was added in the extract for removing the salts and extracted with 100 mL of diethyl ether by three times. The diethyl ether phase was evaporated to dryness and the residue dissolved in 10 mL methanol. Among them, 1 mL of methanol solution was re-evaporated together with 50 μg of orcinol as an internal standard for GC analysis.

5. Supercritical Fluid Extraction

SFE was performed on an Isco supercritical fluid extractor, model SFX 3560 equipped with two Isco 260 D syringe pumps (Lincoln, NE, U.S.A.) using CO_2 (99.9%, Seoul Gas Co. Seoul, Korea) and CO_2 modified with 1, 5, and 10% of methanol, water, methanol containing 10% (v/v) of diethylamine, and water containing 10% (v/v) of diethylamine. The SFE experimental conditions are listed in Table 1. The restrictor temperature was the same as each extraction temperature. The remaining volume was filled with glass wool. In each extraction step, the extract was collected in methanol.

6. GC Analysis

For the measurement of the solubility of pseudoephedrine free base, the extracts in methanol 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 with 1 mg of palmitic acid methyl ester as an internal standard under an N_2 stream, and dissolved in 1 mL of chloroform for GC analysis. SFE extracts of hydrochloride salts of ephedrine derivatives, spiked filter papers, and plant materials were dissolved in 1 mL methanol and transferred into a reaction vial. Orcinol (50 μg) was used as an internal standard and added to each solution and evaporated under an N_2 stream. The extracts were TMS-derivatized with 100 μL of MSTFA at 65 °C for 90 min. GC/FID was performed on a Hewlett Packard (Avondale, PA, U.S.A.) 5890 series II gas chromatograph equipped with an 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 flow rate of 3.7 mL/min. The split ratio was 20 : 1. The oven temperature was increased from 90 (1 min hold) to 124 °C at a rate of 3 °C/min (3 min hold) and then to 280 °C at a rate of 20 °C/min. The injector and detector temperatures were 220 °C and 280 °C, respectively.

RESULTS AND DISCUSSION

Ephedrine derivatives were not extracted at all from the aerial parts of *E. sinica* by pure SCF-CO_2 in the preliminary tests; most alkaloids exist as their salt forms in plant tissues because of their own characteristic basicity [Tyler et al., 1981]. Therefore, the solubilities of these salts should be compared with those of free bases prior to conducting SFE. For this purpose, the solubilities of pseudoephedrine free base and its salts in SCF-CO_2 were investigated by varying temperature (40 and 80 °C) and pressure (13.6-34.0 MPa), and then compared with each other. In the present investigation, the effects of static time and flow rate for the equilibrium state between the solute and solvent were not evaluated, since the purpose of this work was to measure only the effects of various modifiers on the extractabilities of the target compounds. Therefore, although the term "solubility" was used in this study, it may be "pure compound extractability" or "pseudosolubility" in the strict sense, rather than real value of solubility. The solubilities of pseudoephedrine free base in SCF-CO_2 under the conditions employed are shown in Fig. 2. The solubility was increased with increasing pressure at 80 °C, while it did not show any significant enhancement at 40 °C. The solubility of pseudoephedrine in SCF-CO_2 at 80 °C and 34.0 MPa, in which its solubility had the highest value, was determined as 2.47 (±0.22) mg/mL. Differently from these results, however, SCF-CO_2 could extract nothing of pseudoephedrine hydrochloride salt at any experimental condition. These results showed that pure SCF-CO_2 was inefficient for the extraction of the salt forms of ephedrine derivatives in plant tissue. Thus, we intended to use the modifier enhancing the polarity of CO_2 in order to increase the solubilities of ephedrine derivative salts.

For the improvements of solubilities of ephedrine derivative hydrochloride salts, methanol or water as a modifier was introduced into CO_2 at 80 °C and 34.0 MPa by separating syringe pump at the

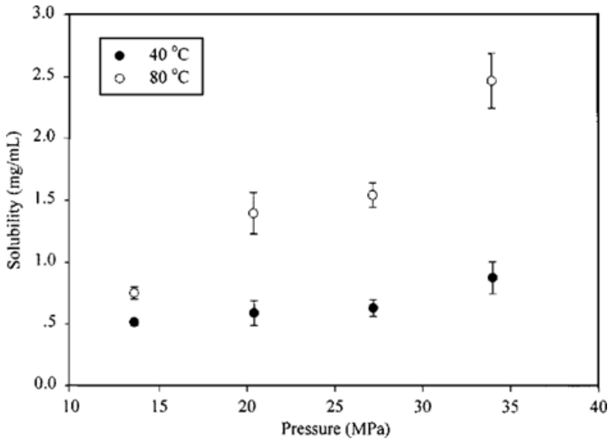

Fig. 2. Solubility of pseudoephedrine free base in supercritical CO_2 at 40 and 80 °C in the pressure range of 13.6-34.0 MPa. All experiments were performed in triplicate.

concentration levels of 1, 5 and 10% (v/v), respectively. The effect of methanol and water is shown in Fig. 3. Water was only efficient for methylephedrine hydrochloride salt, but it had little effect on the other ones. The inefficiency of water may originate from its low miscibility with CO_2. Actually, the addition of water to CO_2 resulted in phase separation (Fig. 4). So, the large volume of water addition may bring about phase separation or aerosol formation with CO_2. In the case of methanol, however, it revealed the significant improvements of the solubilities of the ephedrine derivative salts employed in this study relative to water. Even though the addition of methanol in CO_2 resulted in slight improvements in the solubilities, they were still poor. So, other modifiers to enhance the solubilities of ephedrine derivative salts should be required.

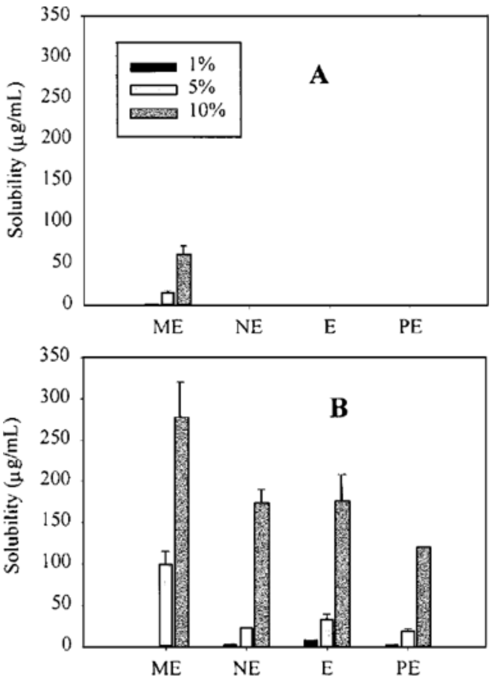

Fig. 3. Effect of water (A) and methanol (B) as a modifier on the solubilities of ephedrine derivative hydrochloride salts in CO_2 at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10%, respectively. ME=methylephedrine; NE= norephedrine; E=ephedrine; PE=pseudoephedrine. All experiments were performed in triplicate.

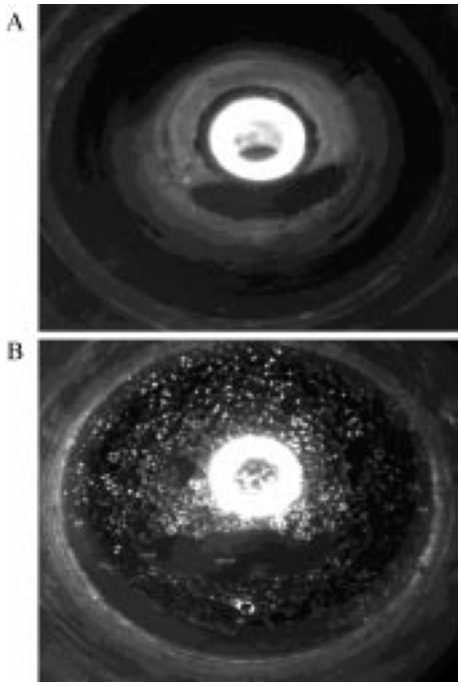

Fig. 4. Comparison of the phase in the mixtures of methanol-CO_2 (A) and water-CO_2 (B) using view cell at 60 °C and 34.0 MPa.

Generally, alkaloidal salts are insoluble in nonpolar solvents, but their free bases are quite soluble in them. Therefore, for solubilizing alkaloids in CO_2, a basified modifier should be introduced to the SFE. For this purpose, diethylamine in water or methanol (10% v/v) was eluted at a concentration of 1, 5, and 10% (v/v). Fig. 5 reveals the solubilities of ephedrine derivative hydrochloride salts in CO_2 mixed with each basified modifier. Except for methyl ephedrine hydrochloride salt, on which the basified modifiers had little enhancing effect relative to neat methanol or water, the addition of modifiers basified with diethylamine largely increased the solubilities when compared to pure methanol or water. In particular, the mixtures of CO_2-methanol-diethylamine (90 : 9 : 1) could more effectively extract these compounds than the other ones employed, in which the solubilities were in the range of 213-564 μg/mL.

In addition to the effect of modifier on solvating power, a modifier also plays very important roles, especially for matrix, where the analyte is strongly bound through chemisroption and physisorp-

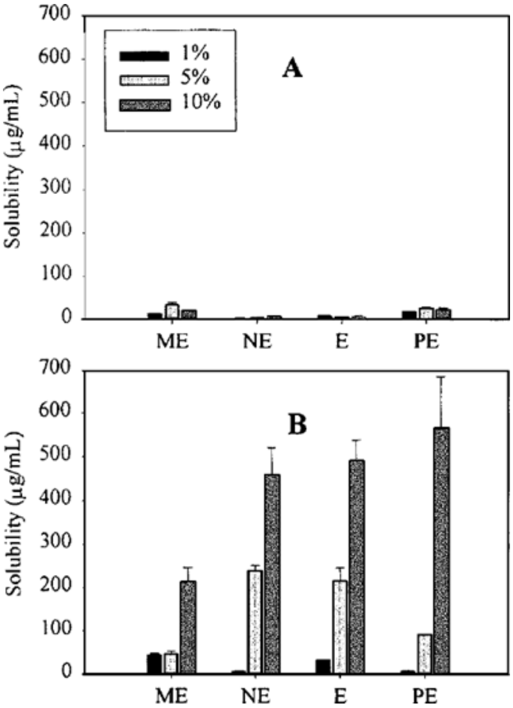

Fig. 5. Effect of water (A) and methanol (B) basified with diethylamine (10% v/v) as a modifier on the solubilities of ephedrine derivative hydrochloride salts in CO_2 at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10%, respectively. ME=methylephedrine; NE=norephedrine; E= ephedrine; PE=pseudoephedrine. All experiments were performed in triplicate.

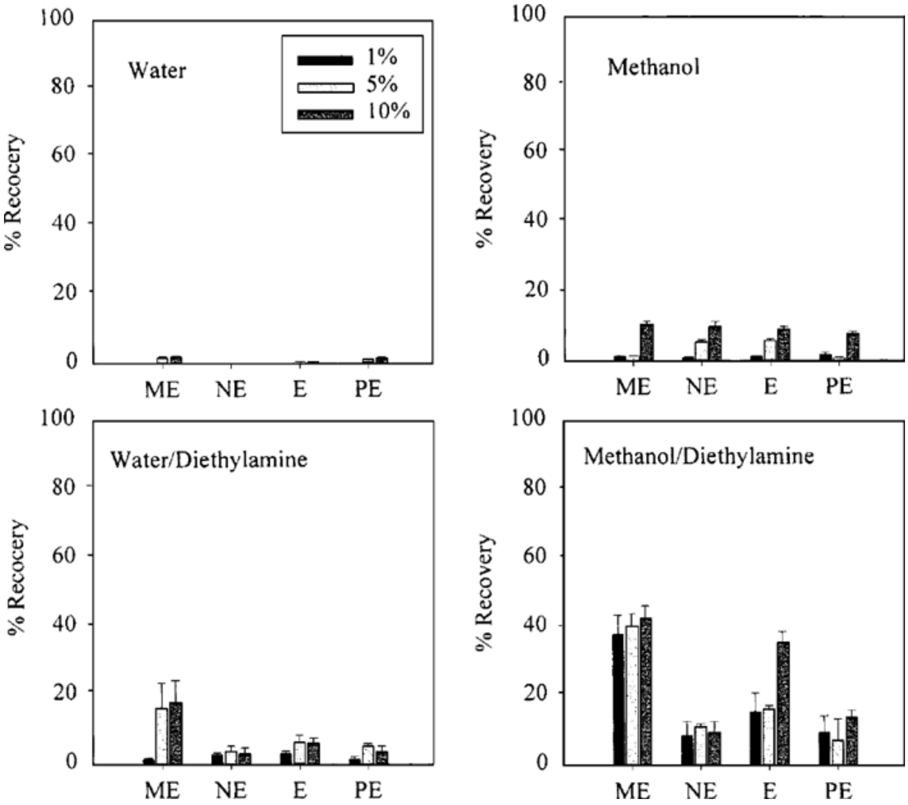

Fig. 6. Effect of the modifiers employed on percent recovery of ephedrine derivatives from filter papers at 80 °C and 34.0 MPa. Concentrations of modifiers: 1, 5, and 10%, respectively. ME=methylephedrine; NE=norephedrine; E=ephedrine; PE=pseudoephedrine. All experiments were performed in triplicate.

tion. Another advantage brought about by using a polar modifier is the swelling of the matrix, thereby increasing the internal volume, which in turn increases the amount of surface area accessible to the near supercritical solvents [Fahmy et al., 1993; Moore and Tyler, 1996]. Therefore, prior to SFE, the effect of the modifiers on a matrix should be evaluated together with that on the solubility. These results could demonstrate that specific interactions occur between ephedrine derivatives and a matrix. In this step, the effects of modifiers were investigated on the SFE extractabilities of ephedrine derivatives from filter papers. Fig. 6 shows the effect of the modifiers on the percent recoveries of ephedrine derivative hydrochloride salts from filter papers. In these results, the modifiers added by diethylamine, that is, diethylamine in water or methanol was more efficient in the extraction of ephedrine derivatives from filter papers than pure water and methanol as shown in solubility measurement. In particular, diethylamine in methanol could extract these compounds by two times as much as any other modifiers tested in this study. Therefore, these results suggested that diethylamine in methanol could be most effective on the SFE efficiencies of ephedrine derivatives by desorption from matrix as well as enhancing the solubilities.

In the test of solubility and desorption from filter papers, diethylamine in methanol as a modifier was found to be the best one for the extraction of ephedrine derivatives. Diethylamine in methanol was obviously effective on the SFE of all the ephedrine derivatives from plant materials as revealed by the results of solubility and desorption from filter papers. In particular, above 80% of methylephedrine could be extracted using 10% of this modifier when compared to a conventional organic solvent extraction. Although diethylamine in methanol among the modifiers was most effective on the SFE efficiencies of ephedrine derivatives from plant materials, the yields of norephedrine, ephedrine, and pseudoephedrine were still below 60% relative to the liquid extraction. Thus, the method to enhance SFE yields of ephedrine derivatives should be performed. For this purpose, extraction time, temperature, and modifier percent were increased. Unfortunately, increase of extraction time and temperature did not show any significant effect on the SFE. However, when adding percent of diethylamine in methanol was increased up to 20%, the extraction yields of ephedrine derivatives were dramatically improved. In the case of methylephedrine and ephedrine, they were completely recovered by SFE using this modifier. The yields of ephedrine derivatives by CO_2 modified with 20% of diethylamine in methanol were compared with those by conventional extraction in Table 2.

In addition to enhancement of the yields, an important result was obtained by this method. Pseudoephedrine, which is the diastereomer (Fig. 1), was extracted only by 44%, while ephedrine was 106%. This might not be caused by the difference of their solubilities but desorption from plant matrix. As shown in Fig. 6, the yield of pseudoephedrine extracted from by CO_2 modified with diethylamine in methanol from filter papers was about half of that of ephedrine, while there was no significant difference in their solubilities. The GC chromatogram obtained by SFE and liquid extraction was provided in Fig. 7. These results show the probability that

Table 2. Yields of methylephedrine (ME), norephedrine (NE), ephedrine (E), and pseudoephedrine (PE) obtained by organic solvent extraction[1] and SFE[2]. Results are mg/g (%RSD)

Extraction method	ME	NE	E	PE
Organic solvent extraction	0.25 (1.2)	0.12 (16.9)	3.22 (6.0)	1.1 (6.0)
SFE	0.37 (0.4)	0.046 (25.1)	3.44 (2.2)	0.40 (8.4)

All experiments were performed in triplicate.

[1]: mixture was CO_2-methanol-diethylamine (80 : 18 : 2), temperature and pressure were 80 °C and 34.0 MPa, respectively, static time was 15 min, total amount of consumed solvent was 50 mL during dynamic extraction.

[2]: extraction solvent was 0.5 M H_2SO_4 followed by basifying with 6 M NaOH and diethylether extraction.

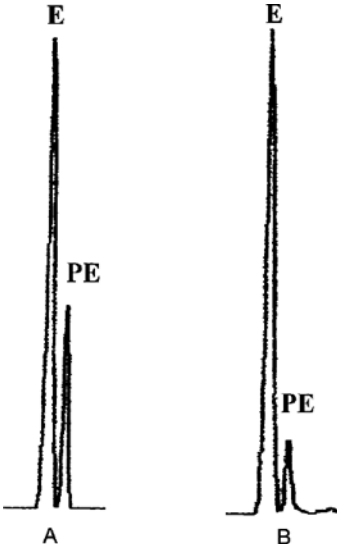

Fig. 7. Comparison of the GC chromatogram obtained by 0.5 M H_2SO_4 extraction (A) and SFE at 80 °C and 34.0 MPa using 20% diethylamine in methanol as a modifier (B). E=ephedrine, PE=pseudoephedrine.

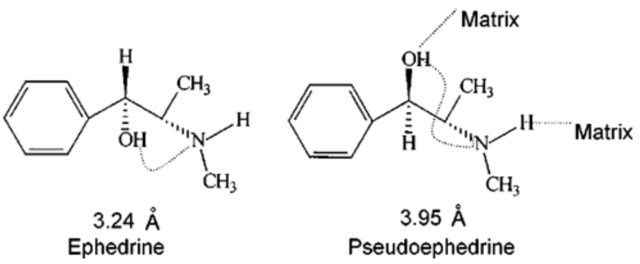

Fig. 8. Proposed change of chemical conformation of ephedrine and pseudoephedrine and distance between hydrogen and nitrogen measured by molecular modeling (Sybyl 6.5, Tripos).

SFE may extract selectively target compound from its stereoisomers. The selective extraction of ephedrine from its stereoisomer, pseudoephedrine, may be due to its structural change of conformation, that is, OH and NH of ephedrine is located in the same plane, so hydrogen of OH and nitrogen may be bound to each other with hydrogen bonding. However, in the case of pseudoephedrine, hydrogens of OH and NH are freer than ephedrine, so they may be bound to matrix. Actually, the results of molecular modeling (Sybyl 6.5, Tripos) supported this proposal. The distance between hydrogen of OH and nitrogen in ephedrine was shorter than that in pseudoephedrine. They were measured as 3.24 Å for ephedrine and 3.95 Å for pseudoephedrine, respectively (Fig. 8).

CONCLUSION

The purpose of this paper was to investigate the effect of modifiers on the extraction yields of ephedrine derivatives. Among the modifiers employed, diethylamine in methanol was the most effective on the extraction of these compounds. The mechanism of this modifier to enhance the SFE yields of the compounds from plant materials was supported by the measurement of solubilities in CO_2 added by the modifiers employed and desorption from filter papers. The improvement of solubilities was found to be caused by changing salts (insoluble in CO_2) of ephedrine derivatives to free bases (freely soluble in CO_2). In addition to increasing the solubilities, diethylamine in methanol as a modifier could greatly enhance the desorption of ephedrine derivatives from a matrix. It is another important result that CO_2 modified with diethylamine in methanol could extract ephedrine more selectively than pseudoephedrine, which is the diastereomer of ephedrine.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Korea Science and Engineering Foundation (KOSEF) for financial support. B.-J. Cha (Dong-A Pharm Co.) for donation of standards.

REFERENCES

Bicchi, C., Rubiolo, P., Frattini, C., Sandra, P. and David, F., "Off-line Supercritical Fluid Extraction and Capillary Gas Chromatography of Pyrrolizidine Alkaloids in *Senecio* Species," *J. Nat. Prod.*, **54**, 941 (1991).

Bugatti, C., Colombo, M. L. and Mossa, A., "Applications of Supercritical Carbon Dioxide to the Extraction of Isoquinoline Alkaloids from *Eschscholtzia californica*," *Planta Med.*, **59**, 626 (1993).

Elisabeth, P., Yoshioka, M., Yamauchi, Y. and Saito, M., "Infrared and Nuclear Magnetic Resonance Spectrometry of Caffeine in Roasted Coffee after Separation by Preparative Supercritical Fluid Chromatography," *Anal. Sci.*, **7**, 427 (1991).

Fahmy, T. M., Paulaitis, M. E., Johnson, D. M. and McNally, M. E. P., "Modifier Effects in the Supercritical Fluid Extraction of Solutes from Clay, Soil, and Plant Materials," *Anal. Chem.*, **65**, 1462 (1993).

Janicot, J. L., Caude, M. and Rosset, R., "Extraction of Major Alkaloids from Poppy Straw with Near Critical Mixtures of Carbon Dioxide and Polar Mixtures," *J. Chromatogr.*, **505**, 247 (1990).

Moore, W. N. and Taylor, L. T., "Extraction and Quantitation of Digoxin and Acetyldigoxin from the *Digitalis lanata* Leaf via Near-supercritical Methanol-modifier Carbon Dioxide," *J. Nat. Prod.*,

59, 690 (1996).

Ndiomu, D. P. and Simpson, C. F., "Some Applications of Supercritical Fluid Extraction," *Anal. Chim. Acta*, **213**, 237 (1988).

Shaeffer, S. T., Zalkow, L. H. and Teja, A. S., "Supercritical Fluid Isolation of Monocrotaline from *Crotolaria spectabilis* using Ion-exchange Resins," *Ind. Eng. Chem. Res.*, **28**, 1017 (1989).

Sugiyama, K., Saito, M., Hondo, T. and Senda, M., "New Double Stage Separation Analysis Method. Directly Coupled Laboratory Scale Supercritical Fluid Extraction Supercritical Fluid Chromatography Monitored with a Multiwavelength Ultraviolet Detector," *J. Chromatogr*, **332**, 107 (1985).

Tang, W. and Eisenbrand, G., "Chinese Drugs of Plant Origin," Springer Verlag, New York, 481 (1992).

Tyler, V. E., Brady, L. R. and Robbers, J. E., "Pharmacognosy," 8th ed.; Lea & Febiger: Philadelphia, Chapter, **8**, 195 (1981).

Zhang, J., Tian, Z. and Lou, Z., "Simultaneous Determination of Six Alkaloids in Ephedrae Herba by High Performance Liquid Chromatography," *Planta Med.*, **54**, 69 (1988).