Journal of Chromatography, 211 (1981) 123–128
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,733

DETERMINATION OF ACONITINE ALKALOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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(Received February 11th, 1981)

SUMMARY

A rapid, specific and precise method using high-performance liquid chromatography has been developed for the separation and quantitation of the aconitine alkaloids. The alkaloid components present in the crude drug "bushi", *Aconitum* roots, have been resolved on an ODS chemically bonded silica gel column using a phosphate buffer (pH 2.7)–tetrahydrofuran (89:11) mobile phase. An alternative method involving ion-pair chromatography with a phosphate buffer (pH 2.7)–tetrahydrofuran (85:15) mixture and 0.01 *M* sodium hexanesulphonate has also been developed. Determination of the aconitine alkaloids by the two techniques showed very good agreement. In terms of speed, component resolution and quantitation, the assay reported here is superior to other methods. The method has been applied to the evaluation of commercially available preparations of aconitine.

INTRODUCTION

The crude drug "bushi" (aconite), prepared from the roots of certain species of *Aconitum* (Ranunculaceae) from China and Japan, is an important material in Oriental medicine and has been used clinically in large quantities in East Asia. Since raw roots of *Aconitum* plants utilized for medicinal purposes contain the aconitine alkaloids and are therefore highly toxic, they are usually processed before use in order to diminish the clinical intoxication. The processing involves hydrolysis of the highly toxic aconitine analogues to the much less toxic benzoylaconine analogues through heating, and loss of the alkaloids by steaming and soaking. However, this procedure is not necessarily standardized so that the alkaloid composition, and consequently the toxicity or the therapeutic effectiveness, of processed *Aconitum* roots varies greatly.

* Part 10 in the Tohoku University series on Pharmaceutical studies on *Aconitum* roots.

0021-9673/81/0000–0000/$02.50

Therefore, a rapid, specific and precise method is required for the simultaneous determination of the aconitine alkaloids in order to evaluate the quality (alkaloid composition and content) of the crude drug "bushi".

A number of procedures have been developed for the analysis of aconitine alkaloids. Recent studies have employed paper electrophoresis[1], thin-layer chromatography[2–4], multi-buffered paper partition chromatography[5] and gas–liquid chromatography[6]. However, for the aconitine alkaloids these techniques are time-consuming, and result in incomplete separations and/or imprecise determinations. In recent years, high-performance liquid chromatography (HPLC) has become an important technique for the analysis of natural products. Despite this, it seems that no author has reported the HPLC analysis of the aconitine alkaloids. We have thus investigated the utility, specificity and sensitivity of HPLC for the simultaneous determination of the aconitine alkaloids. This paper describes the development of an appropriate analytical method and its application to commercially available preparations of aconitine.

EXPERIMENTAL

Reagents and materials

Acetonitrile of HPLC grade and tetrahydrofuran (THF), sodium hydrogen phosphate and phosphoric acid of analytical grade were purchased from Wako (Osaka, Japan). Sodium hexanesulphonate used as the ion-pair chromatographic reagent was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

The mobile phase was degassed in an ultrasonic bath under reduced pressure for 15 min. All the aconitine analogues [mesaconitine (MA), desoxyaconitine (DA), hypaconitine (HA) and jesaconitine (JA)] except for aconitine (A) were prepared from *Aconitum* roots in our laboratory (by extraction, fractionation, chromatography and crystallization). Aconitine was obtained from various companies (Table I).

TABLE I

CONTENTS OF ALKALOIDS IN COMMERCIAL PREPARATIONS OF ACONITINE

Conditions as in Fig. 1A. The results are expressed as the mean (%) of triplicate determinations. – = Not detected.

Manufacturer	*Lot No.*	*HA*	*MA*	*DA*	*A*
Fluka (Buchs, Switzerland)	178794	0.8	–	1.3	94.8
Fluka	191343 76	38.8	58.6	1.1	3.5
Fluka	195652	–	0.8	1.0	97.9
Fluka	817904	38.2	60.5	0.9	3.6
Merck (Darmstadt, G.F.R.)*	Unknown	1.2	17.0	2.1	75.8
Merck**	Unknown	7.5	70.2	0.6	19.0
Nakarai (Kyoto, Japan)	M4N6839	0.4	0.9	–	83.0
Nakarai	V48100	–	1.0	3.6	96.6
Sigma (St. Louis, MO, U.S.A.)	39C-0030	–	–	–	100.0

* Purchased over half a century ago.

** Nitrate.

The benzoylaconine analogues [benzoylaconine (BA), benzoylmesaconine (BMA), benzoyldesoxyaconine (BDA), benzoylhypaconine (BHA) and benzoyljesaconine (BJA)] were prepared from the corresponding aconitines in our laboratory (by heating at 120°C in dioxan–water for 40 min, chromatography and crystallization).

Sample solutions

The aconitine alkaloids were dissolved in 0.1 *M* phosphate buffer (pH 2.7)–acetonitrile (3:2).

Apparatus

All analyses were performed on a Toyo Soda (Tokyo, Japan) Model HLC-803A high-performance liquid chromatograph which was coupled to a stainless-steel column (30 cm × 4 mm I.D.) packed with ODS chemically bonded silica gel (TSK GEL LS 410 ODS SIL, 5 μm; Toyo Soda). The detector was a Toyo Soda Model UV-8 variable-wavelength minitor with a flow cell of volume 8 μl.

RESULTS AND DISCUSSION

Choice of mobile phase

In the analysis of basic substances such as the aconitine alkaloids using a reversed-phase HPLC system of chemically bonded silica gel, it is well known that tailing peaks are obtained due to retention by free silanol groupings when mobile phases such as mixtures of methanol and water are employed. It has been reported that acidic mobile phases (pH 2–3)[7], acidic amine phosphate buffer as eluent[8] or a combination of both[7] can be useful for improving peak shapes and for obtaining improved separations. In the case of the aconitine alkaloids, a method using an ODS chemically bonded silica gel column under acidic conditions gave good peak shapes, as expected. It is also known that the chromatographic behaviour is dependent on the nature of the organic solvent used as the mobile phase, and that the relative retentions vary widely according to the functional groups and structures of the substances when methanol, acetonitrile or THF is utilized[9].

Various solvent systems were, therefore, evaluated for their ability to separate the aconitine alkaloids. Methanol, when used as the organic component of the mobile phase, was found to cause loss of resolution of aconitine and jesaconitine. When acetonitrile was employed, aconitine, hypaconitine and jesaconitine were unresolved, and desoxyaconitine was strongly retained even under the optimum conditions. Similar tendencies were also observed in ion-pair chromatography using sodium hexanesulphonate or sodium perchlorate. When THF was the organic component, the peak shapes were much improved and baseline separations of the ten analogues were achieved. Chromatograms obtained with a mobile phase comprising acetonitrile and THF as the organic components are illustrated in Fig. 1.

The effects on the retention volumes of the aconitine alkaloids of pH and THF concentration of the eluent were examined. Fig. 2 shows the effect of the pH of the phosphate buffer, on eluting with 0.05 *M* phosphate buffer–THF (89:11). The retention volumes of the alkaloids remained relatively unchanged over the acidic pH region. As the pH of the mobile phase was increased from 5, the retentions of certain alkaloids increased. Hypaconitine, mesaconitine and benzoyljesaconine were satis-

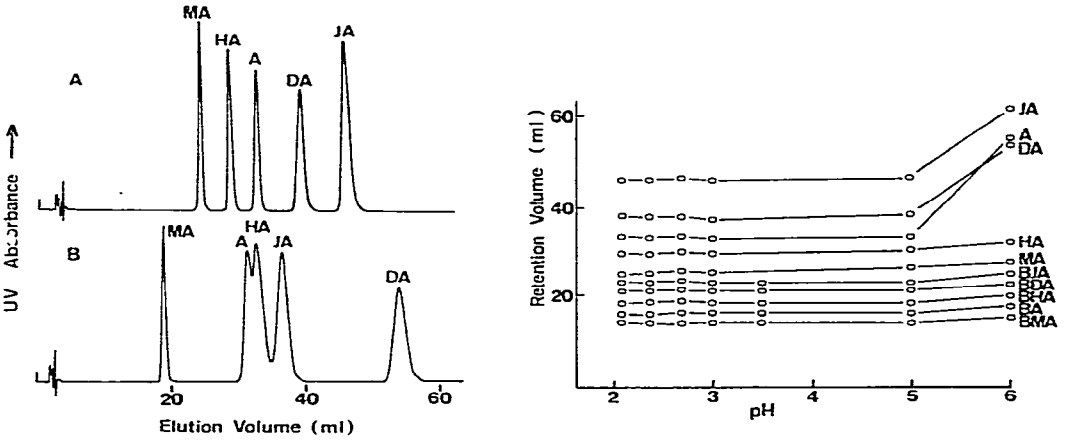

Fig. 1. Solvent selectivity. A: Column, 300 × 4 mm I.D.; packing, TSK GEL LS 410 ODS, 5 μm; mobile phase, 0.05 *M* phosphate buffer (pH 2.7)–THF (89:11); flow-rate, 0.9 ml/min; temperature, 23°C; UV detection at 254 nm; sample size, 20 μl. B: Conditions as in A except that the mobile phase was 0.05 *M* phosphate buffer (pH 2.7)–acetonitrile (70:30). Abbreviations for the aconitine alkaloids are as shown in the text.

Fig. 2. Effect of the pH of the mobile phase on the retention volumes of the aconitine alkaloids. Conditions as in Fig. 1A except for the pH of the 0.05 *M* phosphate buffer.

factorily separated at pH 2.7, where the buffer action was effective; therefore, further chromatography was carried out at pH 2.7. A comprehensive study of the effect of the ratio of the phosphate buffer and THF gave the results shown in Fig. 3. Linear plots of the logarithm of the measured capacity factors of each alkaloid were obtained over the concentration range of 9–15% THF and the order of elution of the alkaloids was unchanged over this range, *viz.*, benzoylmesaconine, benzoylaconine, benzoylhypaconine, benzoyldesoxyaconine, benzoyljesaconine, mesaconitine, hypaconitine, aconitine, desoxyaconitine and jesaconitine. Fig. 4 illustrates a chromatogram of stan-

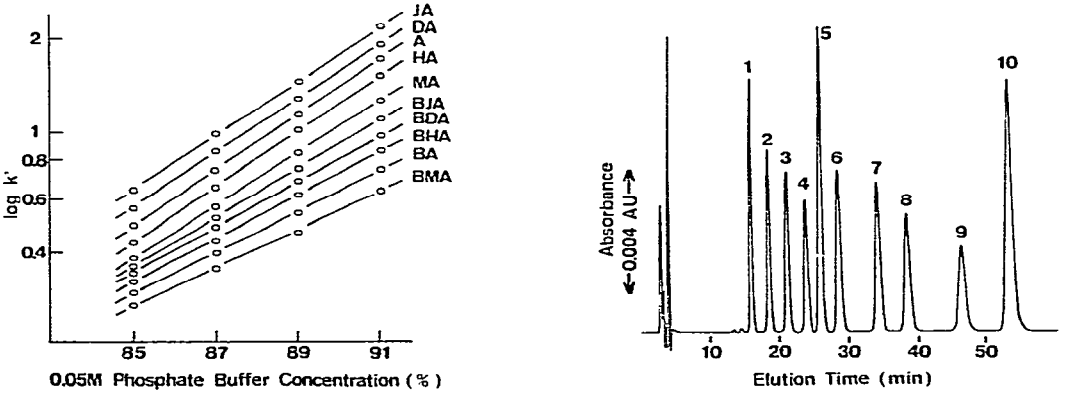

Fig. 3. Effect of the ratio of phosphate buffer and THF in the mobile phase on the retention of the aconitine alkaloids. Conditions as in Fig. 1A except for the ratio of 0.05 *M* phosphate buffer (pH 2.7) and THF.

Fig. 4. Chromatogram of the aconitine alkaloids. Conditions as in Fig. 1A. Peaks: 1 = BMA; 2 = BA; 3 = BHA; 4 = BDA; 5 = BJA; 6 = MA; 7 = HA; 8 = A; 9 = DA; 10 = JA.

dard alkaloids eluted with phosphate buffer (pH 2.7)–THF (89:11), conditions under which optimum separation was obtained.

In the analysis of unknown aconite samples, other constituents may be eluted at the same time as the aconitine alkaloids. Hence, it was considered that the identification of the alkaloids must be substantiated by chromatography using a different separation mode. For this purpose, ion-pair chromatography employing sodium hexanesulphonate was tried. Because addition of the salt increased the retention volumes of the aconitine alkaloids, the ratio of the phosphate buffer and THF was adjusted to 85:15. In Fig. 5 the retention volume of the aconitine alkaloids is plotted against the concentration of sodium hexanesulphonate, and it is seen that the addition of sodium hexanesulphonate increased the retention volumes. The mobile phase having a sulphonate concentration of around 0.01 *M* appeared to offer a satisfactory separation and this was employed in all subsequent assays. A combination of these chromatographic methods is of utility in the analysis of some samples of the crude drug, as will be discussed elsewhere.

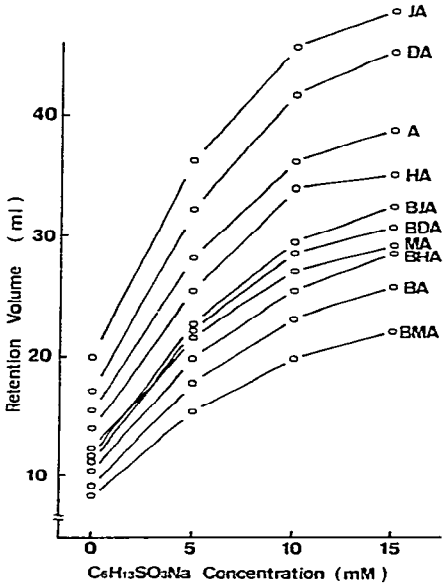

Fig. 5. Effect of the concentration of the ion-pair reagent in the mobile phase on the retention volume of the aconitine alkaloids. Conditions as in Fig. 1A except for that the mobile phase is sodium hexanesulphonate in 0.05 *M* phosphate buffer (pH 2.7)–THF (85:15).

Calibration curves and detection limits

Although aconitine exhibits a UV absorption maximum at 235 nm, the determination of the aconitine alkaloids in the present work was carried out at 254 nm in order that the assay can be done also with a UV detector equipped with a mercury lamp. Sets of standard alkaloids covering the range 0–1.2 μg were run. The calibration graphs were constructed by measuring the peak heights and were computed by linear regression analysis. Calibration graphs based on seven standards are depicted in Fig. 6. Prior conditioning of the HPLC column by running a number of samples ensured stable calibrations and the graphs were linear over the required range. The

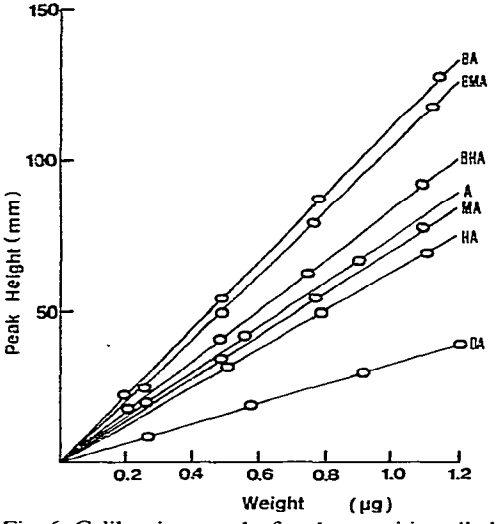

Fig. 6. Calibration graphs for the aconitine alkaloids. Conditions as in Fig. 1A.

coefficients of variation were calculated as 1.52% for mesaconitine and 1.70% for benzoylmesaconine ($n = 11$). Detection limits (signal-to-noise ratio, 3) were found to be 12 ng for mesaconitine and 15 ng for benzoylmesaconine. When the alkaloids were monitored at 235 nm, the sensitivity was about five times as much as that at 254 nm.

Application

Some commercially available preparations of aconitine were subjected to HPLC, using concentrations of *ca.* 0.05 mg/ml. The results are given in Table I. It was found that the purity varied significantly depending on the preparation.

ACKNOWLEDGEMENTS

We are indebted to Professor Shô Itô of Tohoku University, Sanwa Shoyaku Co., Ltd., and Kotaro Kampo Pharmaceutical Co., Ltd., for donation of some commercial preparations of aconitine.

REFERENCES

1 R. R. Paris, G. Dillemann and G. Faugeras, *Ann. Pharm. Fr.*, 17 (1959) 188.
2 K. Tanaka, K. Kawase and S. Takahashi, *Abstracts of the Annual Meeting of the Japanese Society of Pharmacognosy, Kanazawa, 19 September, 1964*, p. 10.
3 H. Zenda, *Kagaku no Ryoiki, Zokan*, 61 (1964) 133–140.
4 F. Kurosaki, T. Yatsunami, T. Okamoto and Y. Ichinohe, *Yakugaku Zasshi*, 98 (1978) 1267.
5 H. Yoshida, S. Kuwano, T. Tamura, Y. Matsunaga and S. Takagi, *Yakugaku Zasshi*, 85 (1965) 709.
6 S. Takagi, T. Katagi and K. Takebayashi, *Yakugaku Zasshi*, 88 (1968) 1242.
7 M. E. F. Biemond, W. A. Sipman and J. Olivié, *J. Liquid Chromatogr.*, 2 (1979) 1407.
8 W. R. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 111.
9 S. R. Bakalyar, R. McIlwrick and E. Roggeudorf, *J. Chromatogr.*, 142 (1977) 353.