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Note

Gas chromatographic determination of ajmaline in the bark of the root of *Rauvolfia vomitoria*

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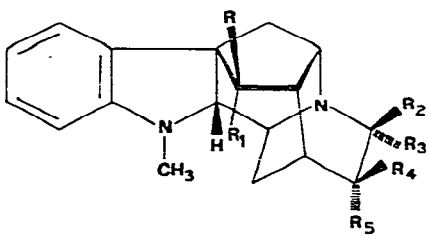
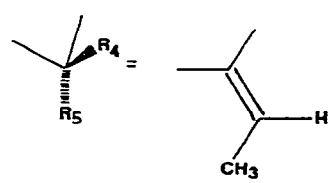
Ajmaline is an alkaloid that is widely used as such or as a precursor of semi-synthetic compounds (17-monochloroacetylajmaline*, N-propylajmaline**), in the therapy of arrythmia[1–6]. The most frequently used source of ajmaline is the bark of the root of *Rauvolfia vomitoria*. The industrial process for its production involves its separation from alkaloids of different basicity (reserpine-like compounds) and final purification from alkaloids with extremely similar structures and basicities (isoajmaline sandwichine, etc.).



Ajmaline	$R = R_3 = OH$	$R_1 = R_2 = R_5 = H$	$R_4 = C_2H_5$
Isoajmaline	$R = R_2 = OH$	$R_1 = R_3 = R_4 = H$	$R_5 = C_2H_5$
Sandwichine	$R_1 = R_3 = OH$	$R = R_2 = R_5 = H$	$R_4 = C_2H_5$
Tetraphyllicine	$R = OH$	$R_2 = R_3 = H$	

* Ritmos Elle; Inverni della Beffa, Milan, Italy.

** Neo-Gilurytmal; Kali-Chemie Pharma, Hannover, G.F.R.

The variety of products present creates considerable difficulties for the determination of ajmaline, which is necessary both for the evaluation of drugs in which it is contained and for quality control during its processing.

Only a few methods have been reported for the determination of ajmaline[7–9], and none of them seems suitable for precise, specific and rapid determinations. To resolve this problem, we have utilized a gas chromatographic technique.

EXPERIMENTAL AND RESULTS

Extraction of the drug and purification of the raw extract

Because of the presence of many products in the raw extract of the drug we developed a method for the purification of samples prior to the gas chromatographic analysis. The principle we exploited was the different basicities of the component alkaloids.

A 5-g amount of the drug (coarse powder) is weighed, transferred to a Soxhlet apparatus for continuous extraction and extracted with 150 ml of methanol for 6 h. The extract is evaporated to about 3 ml and transferred to a separator with the aid of 150 ml of 0.01 *M* hydrochloric acid. The solution is extracted with four 50-ml volumes of chloroform, the combined chloroform extracts are washed with three 50-ml volumes of 0.01 *N* hydrochloric acid and the washings are added to the acidic solution.

The chloroform solution is rejected; 1 ml of 85% phosphoric acid is added and the pH of the aqueous solution is adjusted to 8.5 with 5% sodium hydroxide solution. The aqueous solution is extracted with four 70-ml volumes of chloroform and the combined chloroform extracts are dried with anhydrous sodium sulphate and evaporated to dryness under reduced pressure.

Derivatization

Because of the polarity of the compounds, the sample must be silanized before gas chromatographic analysis, according to the following procedure.

The dry extract of the drug, obtained as described above, is dissolved in 250 ml of chloroform, 1 ml of the resulting solution is transferred to a suitable vial containing 1 ml of the internal standard solution (0.05% arbutin in 1:1 chloroform–methanol) and evaporated to dryness with a stream of nitrogen. Then 250 μl of Trisil Z (Pierce, Rockford, Ill., U.S.A.) are added and the vial is sealed.

The sample is heated at 60° for 1 h, cooled to room temperature and 1 μl is injected under the chromatographic conditions described below. The chromatogram obtained is shown in Fig. 1.

Chromatographic system

The analysis is carried out on a Hewlett-Packard Model HP 5830 gas chromatograph equipped with a flame-ionization detector. The carrier gas is pre-purified dry nitrogen at a flow-rate of 20 ml/min; a 2 m × 3 mm I.D. silanized coiled glass column packed with 3% OV-17 on Chromosorb W HP (100–120) mesh is used. The oven temperature is 270°, injection port temperature 280° and detector temperature 300°. Samples are injected with a 10-μl syringe.

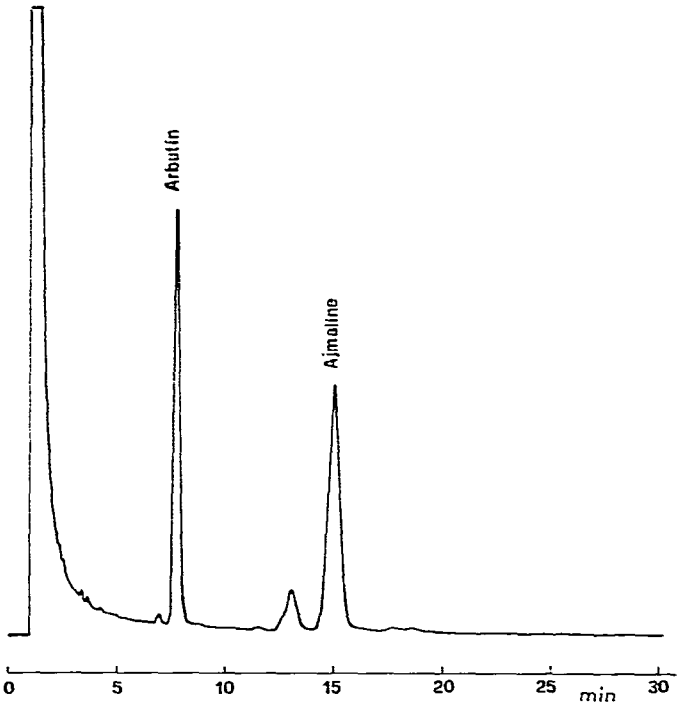


Fig. 1. Gas chromatogram of a sample of *Rauvolfia vomitoria*.

Quantitation

A 1-ml volume of reference solution (0.05% ajmaline in chloroform) is transferred to a suitable vial containing 1 ml of the internal standard solution, evaporated to dryness and silanized as described above. A 1-μl volume is injected.

The calibration data relative to the amount and area of the chromatographic peaks of ajmaline and arbutin are introduced into the integrator, using the internal standard method.

Specificity of the determination

A mixture of isoajmaline, sandwichine, tetraphyllicine, ajmaline and arbutin was analysed under the conditions described. The chromatogram obtained is shown in Fig. 2.

The lack of interference from other products on the chromatographic peak attributed to ajmaline in the drug was also verified by combined gas chromatography–mass spectrometry. The peak was examined by total ion current monitoring; no difference was found in the fragmentation spectrum between pure ajmaline and that analysed in the drug.

Reproducibility and linearity of the chromatographic system

A 100-g amount of ajmaline was analysed ten times following the method described, the results obtained being 99.9, 99.9, 99.6, 102.4, 99.3, 99.1, 98.9, 101.9, 102.0 and 101.5 mg with a mean of 100.45 mg, a standard deviation of 1.35 mg and a

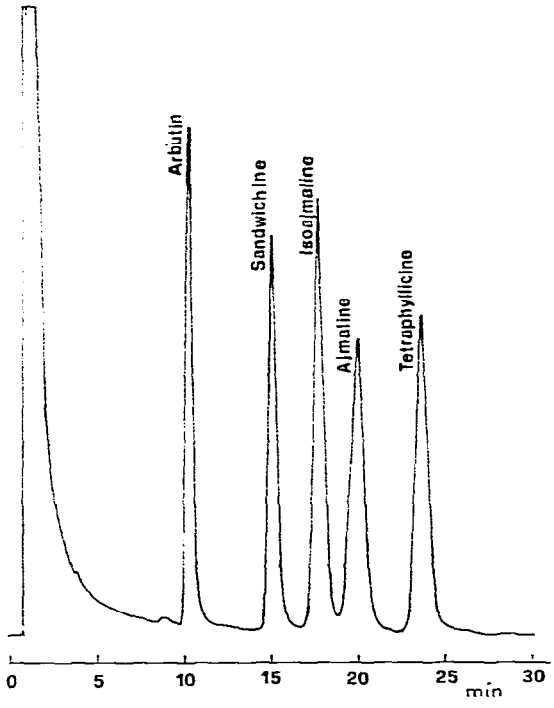


Fig. 2. Gas chromatogram showing the good separation of ajmaline from other alkaloids with extremely similar structures and basicities. These alkaloids can be present in the drug with ajmaline, according to the provenance of the drug.

coefficient of variation of 1.34%. The values obtained demonstrate the good reproducibility of the detection and quantification system.

Different amounts of ajmaline were also analysed, calibration being effected with a sample of medium concentration. The theoretical amounts of ajmaline com-

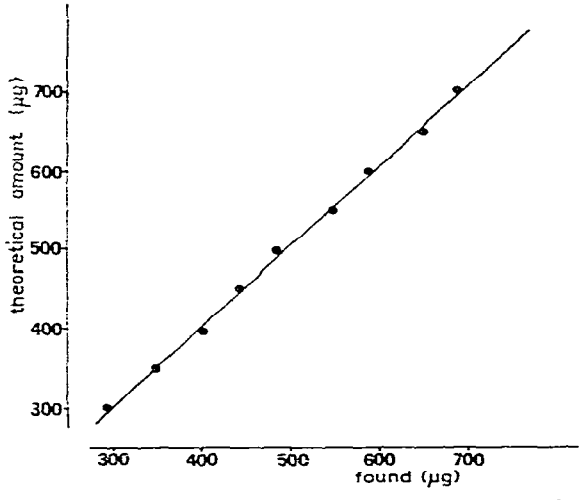


Fig. 3. Linearity of the chromatographic system in the range selected for analysis.

pared with those found experimentally are shown in Fig. 3. In the range selected, which is that normally valid for an analysis, a linear response is evident.

Recovery and reproducibility of the analytical method for the drug

A known amount of ajmaline (100 mg) was analysed five times following the methods of extraction and analysis described for the drug; there was no loss of the product during the preparation of the sample for analysis. The recoveries were: 98.7, 100.9, 99.8, 101.2 and 100.2 mg with an average value of 100.16 mg.

Ten analyses of the same batch of drug were carried out; the results obtained were 2.41, 2.55, 2.55, 2.52, 2.22, 2.41, 2.43, 2.36, 2.44, 2.45% (w/w) of ajmaline with a mean of 2.43% (w/w), a standard deviation of 0.098% (w/w) and a coefficient of variation of 4.05%.

CONCLUSIONS

This method has been used in our laboratories during the last 2 years and has been found suitable for the rapid, specific and precise determination of ajmaline in the bark of the root of *Rauvolfia vomitoria*. It seems that it could also be used for the examination of ajmaline in other drugs; it may also be used in the determination of the other alkaloids reported.

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