Notes

A gas chromatographic determination of pseudo-tropine in tropine

A mixture of tropine and pseudo-tropine, obtained, for instance, by reduction of tropinone, can be analysed fairly simply by the infrared spectrophotometric method of BECKETT et al.[1]. As, however, the absolute accuracy as stated by the authors is ± 3 %, this method is less reliable if the pseudoisomer content to be determined in the tropine sample is as low as 1 %.

The present paper reports a rapid gas chromatographic method for the analysis of mixtures of tropine and pseudo-tropine, with an absolute accuracy less than 0.5 %. While we were preparing the publication SEKERA AND SEKERA[2,3] published their findings of an electrophoretic method for the separation and estimation of these two isomers, which is also very accurate.

Apparatus

The analysis is performed with the aid of a Pye Argon Chromatograph provided with an ionisation detector with Ra-D source and a glass column of 1.20 m × 4 mm I.D.

The packing material used is Chromosorb P (175–210 μ), which is washed 3 times with half-saturated methanolic alkali, decanted and evaporated to dryness in a Rotavapor*.

The stationary phase, Apiezon L, is dissolved in chloroform and mixed with the support. The resulting mass is similarly evaporated to dryness in a Rotavapor, and allowed to dry overnight in a vacuum oven at 150°.

After the column has been filled, it is conditioned at 200° for several 24-h periods in a moderate argon flow in a separate oven. This oven was constructed in order to avoid unnecessary operation of the gas chromatograph and to protect the detector from early soiling; it permits the conditioning of new columns as well as the storage of unused ones. Conditioning must be continued until resolution is adequate.

Results and discussion

Three different column packings were tried out, in an attempt to obtain complete separation of both isomers. The first trials with a column of 7 % (w/w) Apiezon L on Chromosorb P treated with methanolic alkali, were unsatisfactory since the peaks were highly asymmetrical ("tailing"). A column with the same percentage of stationary phase, on a support pre-treated with HCl and soda, as recommended by GOLDING et al.[4] for N-bases, did not produce better results. The tailing vanished completely on increasing the stationary phase to 15 % on Chromosorb P, prepared as indicated under Apparatus.

Any tropinone traces left the column first, followed by tropine and pseudo-tropine

* Rotavapor: Firma Büchi, Flawil, Switzerland.

(Fig. 1). The KOVÁTS retention indices[5], found for this basic Apiezon column, are 1230, 1269 and 1340 respectively. The column, which has ± 1500 theoretical plates for tropine, has a high thermal stability; it has been in use for nearly two years.
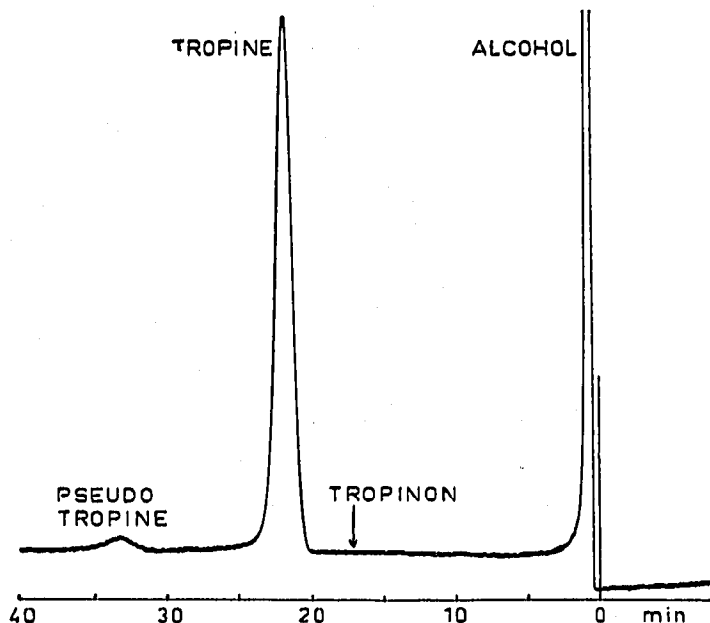

Fig. 1. Gas chromatogram of a tropine sample containing 5.6% pseudo-tropine. Apparatus: Pye Argon Chromatograph with 1.20 m × 4 mm I.D. glass column. 15% Apiezon L on basic Chromosorb P (175-210 μ). Temperature: 132°; gas velocity: 40 ml/min. Detector voltage: 1500 V. Sample: 0.2 μl of an alcoholic solution of tropine (10 mg/ml).

Quantitative determination

Among the usual methods for the quantitative interpretation of chromatograms the cut-and-weigh method is the only suitable one, if tropine samples with a pseudo content between 0 and 10 % are concerned.

The method advocated by PECSOK[6], however, which apparently has not been commonly accepted, also gives quick and accurate results[7,8]. It is based on the principle of the linear relation between the standard deviation σ of a normal distribution curve and the retention time t_R[9]. It is always more accurate to determine the latter value rather than the much lower σ value.

The area under a Gauss curve is given by:

$$A = 2.507\, h\, \sigma$$

where h is the peak height, so that with the relation $\sigma \sim t_R$ the product $t_R \times h$ represents the area formed by the curve. From the $t_R \times h$ values of both peaks the pseudo content may provisionally be determined from the formula

$$\text{percentage} = \frac{t_R \times h}{\Sigma(t_R \times h)} \times 100$$

The exact content is found from a calibration curve obtained by preparing a series of mixtures of the gas chromatographically pure components and plotting their $t_R \times h$

percentages *versus* the actual contents. Fig. 2 depicts such a curve, which holds for the column used under the conditions of temperature and gas rate mentioned. The S-shaped calibration curve intersects the 45°-line exactly at the point where isomer ratio and retention time ratio equal each other.
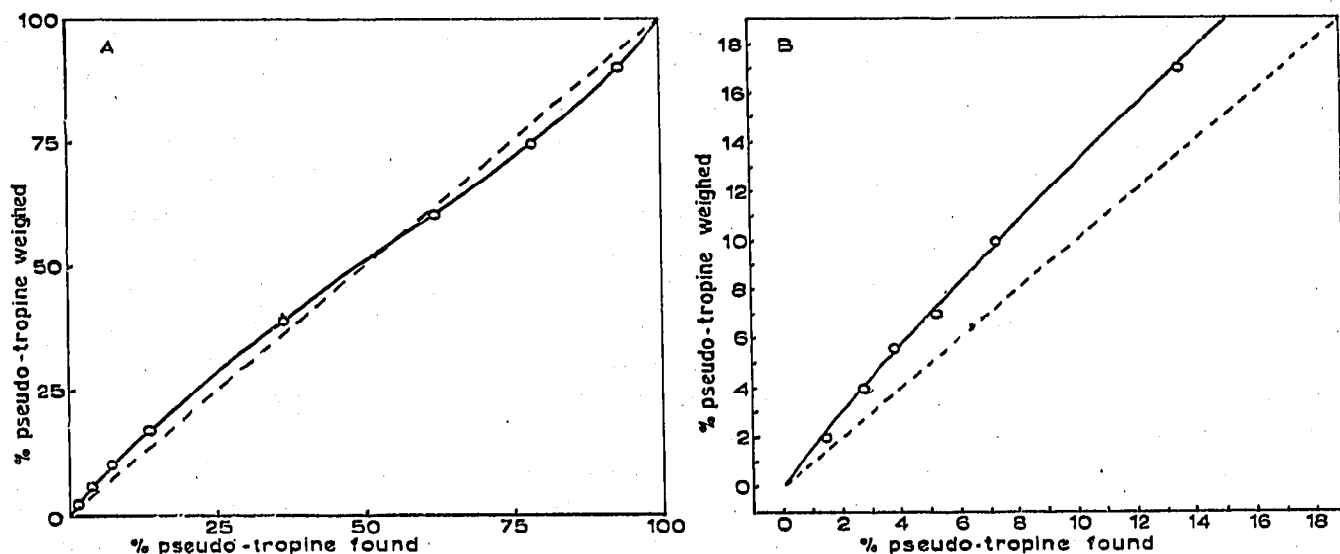

Fig. 2. True pseudo-tropine percentages *versus* those found by the present gas chromatographic method (Fig. 2 B is a detail enlargement of fig. 2 A).

Since measurements have proved that the standard deviations of the peaks for both components are independent of the amount of sample, the shape of the curve must probably be attributed to the non-linear nature of the detector response in this region ($0.02-2\gamma$ of substance). On re-evaluation of the curve only a small correction proved to be necessary after one year.

Analysis of mixtures of tropine and pseudo-tropine of known composition never revealed an absolute deviation exceeding 0.5 % of the pseudo content.

Research Department of N.V. Koninklijke Pharmaceutische   C. VAN DER VLIES
Fabrieken Brocades-Stheeman en Pharmacia*,   B. C. CARON
Amsterdam (The Netherlands)

[1] A. H. BECKETT, N. J. HARPER, A. D. J. BALON AND T. H. E. WATTS, *Tetrahedron*, 6 (1959) 319.
[2] A. SEKERA AND A. SEKERA, *21e Congresso di Scienci Farmaceutiche Pisa*, 1961, p. 816-819.
[3] A. SEKERA, *Ann. Chim. (Paris)*, 7 (1962) 537.
[4] W. E. GOLDING AND A. C. TOWNSEND, *Chem. Ind. (London)*, (1960) 1476.
[5] E. KOVÁTS, *Z. Anal. Chem.*, 181 (1961) 351.
[6] R. L. PECSOK, *Principles and Practice of Gas Chromatography*, John Wiley and Sons, New York, 1959, p. 145.
[7] K. K. CAROLL, *Nature*, 191 (1961) 377.
[8] H. WEISS AND A. KREYENBUHL, *Bull. Soc. Chim. France*, (1961) 603.
[9] J. C. BARTLET AND D. M. SMITH, *Can. J. Chem.*, 38 (1960) 2057.

* Looiersgracht 27-39, Amsterdam.