Journal of Chromatography, 324 (1985) 475–479
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 624

Note

Gas–liquid chromatographic determination of azintamide (Ora-gallin) in pharmaceutical formulations

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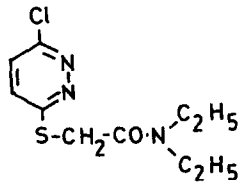
(Received February 5th, 1985)

Some compounds, such as the salts of steroidal bile acids, stimulate the secretory activity of the liver in promoting the flow of hepatic bile[1]. Although they are used to promote the intestinal absorption of food fats, bile salts and some other substances also act as choleretics, *i.e.*, increase the output of bile by the liver. Such compounds are sometimes used singly or in combination with each other to treat the symptoms of fatty infiltration of the liver by encouraging the removal of, or decreasing the deposition of, liver fat and appear to reduce cholesterol levels in the blood and liver.



The potent choleretic drug azintamide, 2-[(6-chloro-3-pyridazinyl)thio]-N,N-diethylacetamide, was synthesized[2,3] and registered under the trade-name Ora-gallin* (ST9067). The choleretic and pharmacological characteristics of azintamide have been studied in patients[4], and its toxicological properties were observed in experimental animals[5]. Owing to its potent choleretic action and moderate cholepoetic activity, the drug is dispensed in some galenical formulations for the therapy of fatty indigestion, cholangitis, cholecystitis, icteric and posticteric conditions. It can also be used for liver protection in cases of cholecystopathy, meteorism and hepatogenic dermatosis.

Spectrophotometric methods for the determination of azintamide either in the pure form or in galenicals have been described[6]. No official method for the analysis

* Pure form; Österreichische Stickstoffwerke, Linz/Donau, Austria. Soragallin is the trade-name of azintamide tablets manufactured by Misr Company for Pharmaceutical Industries, El-Mataria, Cairo, Egypt.

0021-9673/85/$03.30

of this drug has been reported, although there are a few reports on its physical and physico-chemical properties[7,8].

In this paper, a simple and accurate gas–liquid chromatographic (GLC) method for the quantitative determination of azintamide in authentic samples and pharmaceutical formulations is described.

EXPERIMENTAL

Apparatus

A Pye Series 104 gas chromatograph, equipped with a flame ionization detector was attached to a Unicam AR 25 linear recorder.

The optimal chromatographic conditions were as follows. A 5 ft. × 4 mm I.D. glass column was packed with 10% Silar on 100–120-mesh Diatomite C AW and operated isothermally at 250°C. The detector temperature was 300°C, the chart speed 2 min/cm (at 10 mV normal sensitivity), the attenuation 1 × 10^4, backing-off range 1 ×, and the volume injected 5 μl. The carrier gas was N_2 at 3 kPa/cm², flow-rate 42 cm³/min; other gases were air at 2 kPa/cm² and hydrogen at 2 kPa/cm².

Chemicals and reagents

Azintamide. A reference authentic sample of Ora-gallin was kindly provided by Dr. S. A. Ismaiel, General Director of Research Directorate, Misr Company for Pharmaceutical Industries. The sample was utilized as supplied, without further treatments. The melting range was 97.5–98.5°C, determined in a capillary tube according to the BP (1973)[9].

Soragallin tablets. Sugar-coated tablets each containing 100 mg of azintamide, Batch No. 122081, were purchased in local pharmacies.

Chloroform. Chloroform from Prolabo (Paris, France) was redistilled prior to use.

Preparation of calibration graph

A series of eight standard solutions of authentic azintamide were prepared for GLC analysis by dilution with chloroform of a 5 mg/ml stock solution of the drug in chloroform to give concentrations ranging from 0.5 to 3.0 mg/ml. Triplicate injections of 5 μl each on to the column were made and the average results of the area counts were plotted against concentration.

Preparation of samples

Twenty-five azintamide tablets (Soragallin) were weighed accurately and the average weight of a tablet was calculated. The tables were pulverized and an amount of powder containing about 50 mg of azintamide was weighed accurately and transferred quantitatively into a 25-ml volumetric flask. About 20 ml of chloroform were added and the mixture was shaken for about 5 min, then diluted to the mark with the same solvent. If necessary, the suspended solution was centrifuged at about 3500 rpm for about 5 min before injecting an aliquot of the clear solution of the drug.

The concentrations of azintamide in the chloroform extract of the powdered tablets were determined directly from the calibration graph.

Recovery experiment

An accurately weighed amount of pure azintamide equivalent to about 50% of the labelled amount in tablets was added to an accurately weighed amount of powdered Soragallin tablets, followed by the above procedure for preparing the samples.

UV spectrophotometric method[6]

The $A_{1\,cm}^{1\%}$ value of azintamide solution in ethanol was determined at 258 nm ($\lambda_{max.}$) and was found to be 547. A sample was prepared as described above but with extraction with absolute ethanol (Uvasol, E. Merck, Darmstadt, F.R.G.) instead of chloroform. The absorbance (A_a) of the unknown sample was measured against a blank using a 1-cm cell. The concentration was calculated from

$$C\ (\text{mg-\%}) = \frac{A_a}{0.547} \cdot 100$$

RESULTS AND DISCUSSION

Several different GLC columns, *e.g.*, dinonyl phthalate (DNP) (10%) on Diatomite C AW, sylenyl phosphate (XPH) (5%) on Gas-Chrom Q, squalane (10%) on

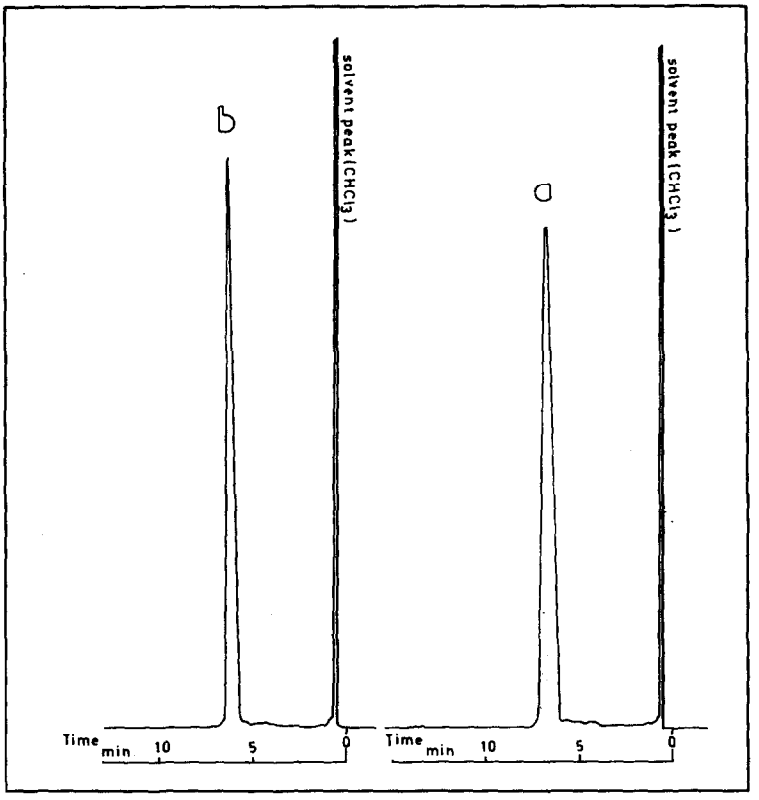


Fig. 1. Typical chromatograms of 5-μl injections of (a) 3 mg/ml solution of an authentic azintamide sample in chloroform and (b) an amount of powdered tablets equivalent to 2 mg/ml of azintamide extracted in chloroform.

TABLE I

COMPARISON OF GLC DETERMINATION OF AZINTAMIDE IN TABLET FORM (SORAGALLIN) WITH A DIRECT UV SPECTROPHOTOMETRIC PROCEDURE

The purity of the authentic azintamide sample was verified by the proposed GLC procedure and was found to be 98.91 ± 0.87%.

GLC			*UV spectrophotometry (258 nm)*	
Stated amount (mg)	*Amount added (mg)*	*Recovery (%)*	*Stated amount (mg)*	*Recovery (%)*
100	–	97.50 ± 1.27* (n = 6)	100	98.63 ± 1.43* (n = 4)
			Student's t = 1.498 (2.571)**	
–	50	98.77 ± 1.11* (n = 4)	–	–

* Standard deviation for n experiments.

** The figure in parentheses is the corresponding theoretical t-value at $n - 1$ degrees of freedom.

Diatomite C AW, Silar (10%) on Diatomite C AW and silicone oil (5 or 10%) on Gas-Chrom Q, were tried in order to select the most appropriate one for the GLC separation of azintamide. Silar (10%) on 100–120-mesh Diatomite C AW proved to be the most efficient. There is no need for a preliminary drug separation or sample clean-up, and also it is not necessary to prepare a derivative of azintamide. A complete assay of azintamide in a sample of tablets requires about 15–20 min; the retention time is about 8 min. Satisfactory peaks were obtained (Fig. 1) when the column was maintained isothermally at 250°C, a linear relationship being obtained at concentrations in chloroform in the range 0.5–3.0 mg/ml. To maintain this linearity, the peak height of the sample should at least about 5% of that of the solvent peak. Tablet components such as diluents, water-soluble colouring matter in the sugar coating and excipients do not interfere in the proposed GLC method because of their insolubility in the solvent used for extracting the drug.

Table I gives the results obtained in comparison with those obtained by a direct UV spectrophotometric method[6]. Good mean recoveries were obtained for pure azintamide (98.91 ± 0.87% compared with the stated amount of the drug in Soragallin tablets of 97.50 ± 1.27%) and for an additional 50% of azintamide added to the stated amount in tablets (98.77 ± 1.11%). A t-test at the 95% probability level showed that there are no significant differences between the proposed method and the direct UV spectrophotometric method, and also the variances indicated no significant differences in the precisions of the two methods.

It can be concluded that the proposed method for the quantitative determination of azintamide either in pure form or in pharmaceutical formulations is simple, accurate and can be used as a stability-indicating method.

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