Journal of Chromatography, 166 (1978) 310–312

CHROM. 11,201

Note

Determination of histamines in wines and musts by reversed-phase high-performance liquid chromatography

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(Received May 31st, 1978)

As little as 8 mg of intravenously injected histamine can elicit an undesirable physiological response in humans[1]. Although it is poorly absorbed when taken orally, the occurrence of up to 30 mg/l in some wines[2] has prompted the search for a rapid histamine determination. Column chromatography[3], thin-layer chromatography[4], ion-exchange chromatography using a fluorescent derivative[5], gel electrophoresis[6], use of an amino acid analyzer[7] and other physiochemical methods[2] have previously been employed and their specificity and sensitivities have recently been reviewed[8].

In this paper we describe a fast acceptable method for determining histamine concentrations in wines and musts.

EXPERIMENTAL

A 5-ml aliquot of wine was passed through a Sep Pak C18 cell to remove pigments and other column-contaminating substances. After the pre-column clean-up an *ortho*-phthaldialdehyde (OPT)–histamine derivative was obtained by mixing an equal part of wine and a solution of 2.5% (w/v) boric acid and 0.002% mercaptoethanol, titrated with NaOH to a final pH of 10.40 $\pm$ 0.02 and to which 10 ml of methanol containing 600 mg OPT (Durham, Palo Alto, Calif., U.S.A.) was added. A 10-μl sample of this solution was placed in a Waters U6K 1-ml injector loop on a Waters ALC/GPC-254 liquid chromatograph. The column used was a μBondapak C_{18} column with volume 2.2 ml. The mobile phase consisted of 40% acetonitrile in water buffered to pH 7 with K_2HPO_4; the flow-rate was 2.0 ml/min at 2000 p.s.i. and the temperature was ambient. Two Waters Model 450 variable-wavelength detectors were used at 200 nm (0.005 a.u.f.s.) and 220 nm (0.01 a.u.f.s.).

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RESULTS AND DISCUSSION

A large number of reversed-phase solvents including phosphate buffers, methanol–acetic acid and methanol–phosphate systems were tried in order to specifically increase the retention time for histamine for underivatized or direct observation. As the resolution of the histamine peak parameters was inacceptable, the OPT derivative was used. The OPT derivative is fluorescent and although the fluorescent detector does give more precise data, the more mechanically reliable UV absorbance detectors were used, because the degree of accuracy required in most cases is $\pm 10\%$.

Empirically it was determined that the histamine peak was best resolved at 200 nm absorbance while a second absorbance at 220 nm was used to confirm changes in elution pattern. The column volume was 2.2 ml and the retention volume for histamine was 15.0 ml, tending to decrease to 14.7 ml during the course of the analyses. This problem was corrected by flushing the column with 1 ml of acetonitrile after every third determination.

Quantitation

The data presented in Figs. 1 and 2 indicate that the relationship between histamine content and peak height is linear over the concentration range studied and that the minimum detection was 0.015 μg.

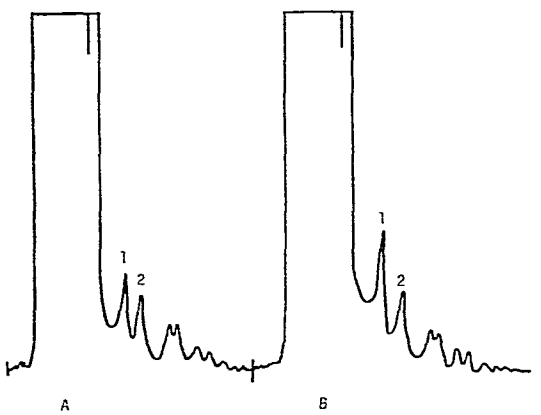

Fig. 1. Chromatograms of diluted wine samples. 1 = Histamine; 2 = unidentified compound. (A) a 1:1 dilution of sample wine in water to which no histamine has been added; (B) the same dilution to which 2.1 μg (1 ml) of histamine has been added.

Although the data are not presented, the concentration/peak height relationship was valid for 210 and 280 nm absorption indicating that it is unlikely that other wine constituents are co-eluting with the histamine. The data in Fig. 1 are from a sample of white dry (0.2% residual sugar) table wine. In subsequent experiments histamine concentrations in dry and sweet reds and in musts were analyzed. As sugar alters the histamine peak baseline, the sweeter wine and must determinations are less reliable.

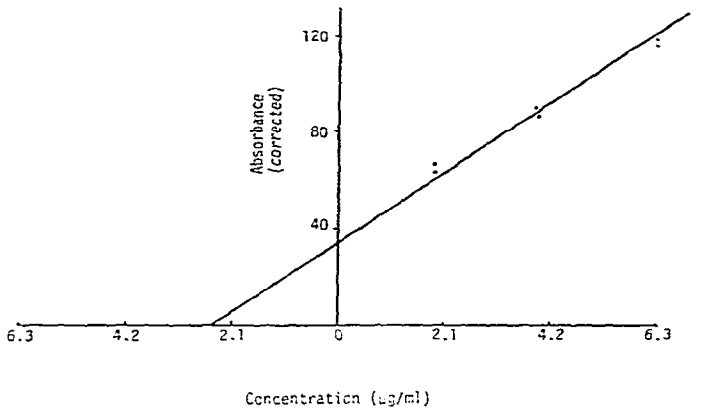

Fig. 2. Working curve for histamine additions (Fig. 1, method 5). Concentrations are averages of 2 determinations. Differences in sample volume were corrected by reference to peak 2, an invariant internal standard.

Although greater accuracy can be obtained by integrating the area under the curve the peak height was easier to mesure and afforded a degree of accuracy acceptable for most studies.

The method described is simple, quick and inexpensive, giving good routine repeatability for the histamine content of wines and musts.

ACKNOWLEDGEMENT

We are grateful to Dave Cotter of Waters Assoc. for the use of instruments and assistance with HPLC protocol. Financial assistance of National Research Council of Canada Grant A6308 and Wine Institute Grant 3602 is acknowledged.

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