Journal of Chromatography, 244 (1982) 159–165
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,906

Note

Isovaleronitrile, a characteristic component of beet molasses alcohol

J. STRATING* and W. M. WESTRA
Heineken Technisch Beheer BV, P.O. Box 510, 2380 BB Zoeterwoude (The Netherlands)
(First received March 5th, 1982; revised manuscript received March 17th, 1982)

Grain alcohol and beet molasses alcohol can both be used for the manufacture of alcoholic beverages. Within the E.E.C. there is a price difference between these two sources, which together with the fact that no method is available to distinguish them, could give rise to malpractices.

If molasses alcohol were to contain a component not present in grain alcohol it would be possible to discriminate the two types of alcohol. The present paper describes a method[1] based upon the determination of the component isovaleronitrile (IVN) present in beet molasses alcohol but virtually absent in grain alcohol.

EXPERIMENTAL

Materials

Silica gel without binder was obtained from Camag (Muttenz, Switzerland). Sodium chloride, *n*-pentane, *n*-hexane and dichloromethane were purchased from E. Merck (Darmstadt, G.F.R.). Heptanone-2, the internal standard, was obtained from Fluka (Buchs, Switzerland) and Sudan Red III from BDH (Poole, Great Britain). Isovaleronitrile (IVN) was a gift from the Central Institute for Nutrition and Food Research (Zeist, The Netherlands).

The glass capillary columns, 50 m × 0.5 mm I.D. (Carbowax 400) and 100 m × 0.5 mm I.D. (SE-30), were supplied by R.S.L. (Gent, Belgium). The six-port micro volume Carle valve was obtained from Techmation (Schiphol, The Netherlands).

Standard solutions

(1) A solution of 0.01 % (w/v) Sudan Red III in *n*-pentane
(2) 15 μl heptanone-2 in 100 ml ethanol
(3) 5 μl IVN in 100 ml ethanol

Gas chromatography

The GC system[1] consisted of two glass capillary columns (Carbowax 400) connected to a six-port micro volume valve mounted in an oven of a Carbo Erba Fractovap 2301-AC, dual FID. Operating conditions were: carrier gas, nitrogen; flow-rate, 7.5 ml/min (Carbowax 400 column), 4.8 ml/min (SE-30 column); oven temperature, 57°C; injection port temperature, 150°C.

0021-9673/82/0000–0000/$02.75

Method

A 250-ml diluted alcohol sample (alcohol strength 10%) to which *ca.* 70 ng NaCl and 10 μl internal standard solution had been added was extracted with two 25-ml volumes of pentane. The total pentane extract was washed with 17 ml saturated NaCl solution and a volume of 42 ml pentane was usually recovered. After addition of 4 μl of Sudan Red solution, the washed pentane extract was subjected to adsorption chromatography on a silica gel column[1].

The silica gel used for the adsorption chromatography was washed with diethyl ether and methanol, dried and heated for 6 h at 150°C. Immediately after cooling to 50°C, it was stored in hexane (*ca.* 75 g silica gel in 250 ml hexane). The column was made from a polypropylene syringe (4.5 mm I.D.), cut off to a length of 6.5 cm, measured from the Luer tip of the syringe. A small plug of Kleenex tissue-paper was inserted in the Luer tip and the column was slurry packed with the hexane–silica gel mixture under a nitrogen pressure of about 2 bar. After packing, the column was covered with a disk of filter-paper (White band). The Luer tip end of the column was connected to a stainless-steel reservoir filled with the washed pentane extract to which Sudan Red solution had been added. Under nitrogen pressure of about 2–3 bar, the pentane extract was forced through the silica gel column.

A red-yellow band formed on the column indicated the IVN fraction. To collect this fraction the column was disconnected from the pentane-reservoir, and connected by means of a simple stainless-steel fitting pressed in the opposite open end of the column to a small stainless-steel reservoir containing 2 ml dichloromethane. The band of Sudan Red formed on the column was now back-flushed with dichloromethane under a slight nitrogen pressure of 0.8 bar. The moment this band reached the Luer tip end of the column, 300 μl dichloromethane were collected and carefully concentrated to *ca.* 10 μl.

A 1-μl volume was injected on the Carbowax 400 column. The IVN fraction emerging from this column between 17′20″ and 18′50″ was switched on-line to the SE-30 column. The exact switching parameters were obtained by injection of a dilute solution of IVN on the Carbowax 400 column.

RESULTS AND DISCUSSION

The method to determine traces of 2-*trans*-nonenal in beer[1] was adapted for use in this investigation on beet molasses alcohol *versus* grain alcohol. In cooperation with the Central Institute for Nutrition and Food Research of the Dutch Organisation for Applied Scientific Research (TNO) in Zeist, a component found in beet molasses alcohol but not in grain alcohol, was identified as isovaleronitrile (IVN) by means of gas chromatography–mass spectrometry. Fig. 1 shows the mass spectrum of IVN.

The quantitation of IVN was only possible by means of multidimensional GC, owing to the admixtures present in beet molasses alcohol (Fig. 2). By switching the fraction (17′20″–18′50″) to an SE-30 column it was possible to separate the IVN from the interfering components (Fig. 3).

An extract of grain alcohol analysed in an identical manner to beet molasses alcohol showed no traces of IVN (Fig. 4).

Finally, Figs. 5 and 6 show chromatograms of extracts of Dutch gin manufactured from beet molasses alcohol and grain alcohol, respectively.

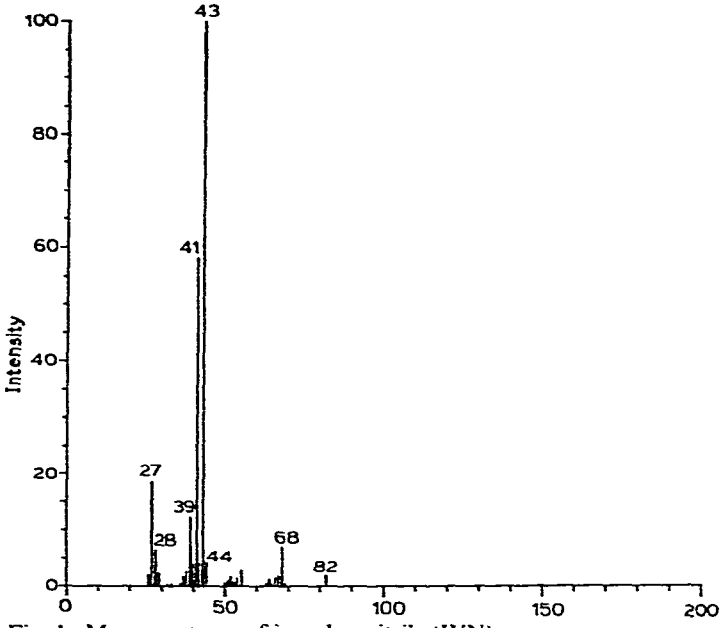

Fig. 1. Mass spectrum of isovaleronitrile (IVN).

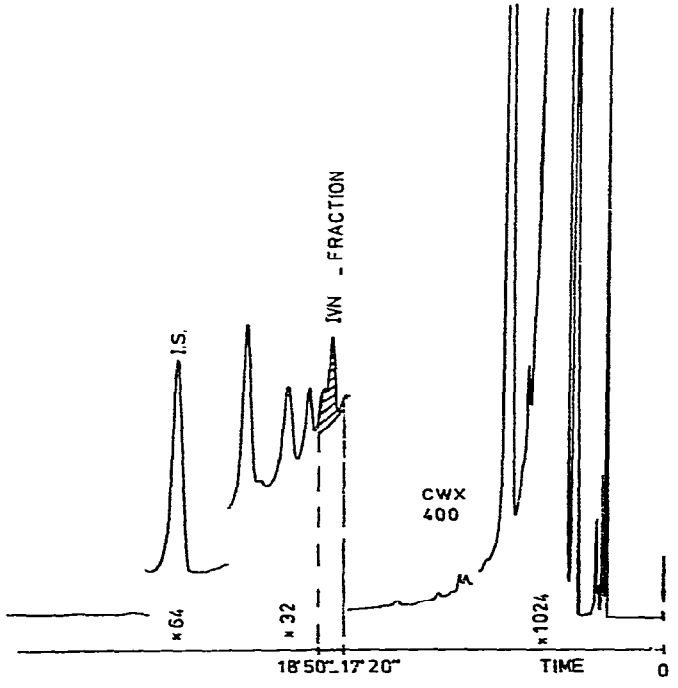

Fig. 2. Gas chromatogram of an extract of beet molasses alcohol on a Carbowax 400 glass capillary column. Conditions described in text. I.S. = Internal standard; IVN = isovaleronitrile.

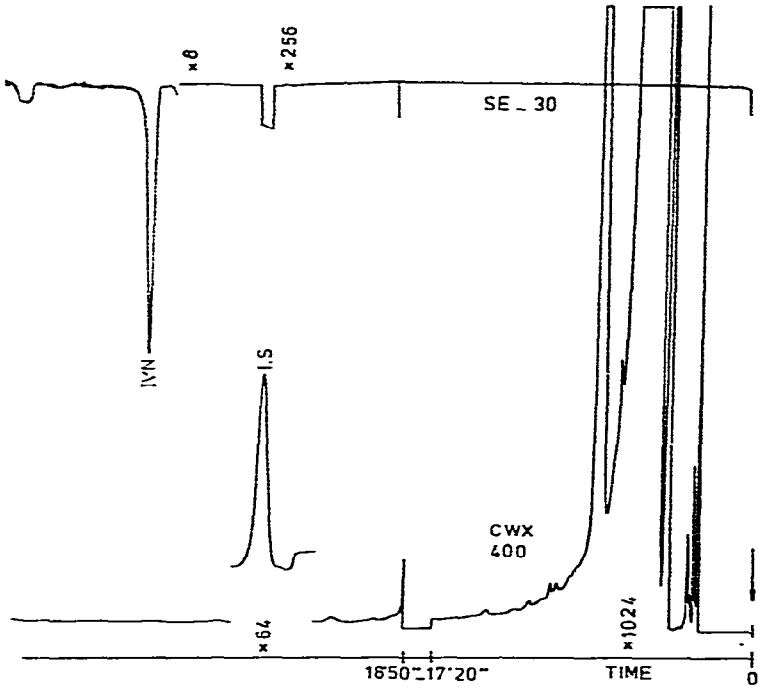

Fig. 3. Gas chromatogram of an extract of beet molasses alcohol on a dual-column system. On-line switching of the IVN fraction (17'20"–18'50") of the Carbowax 400 column to the SE-30 column. Conditions described in text.

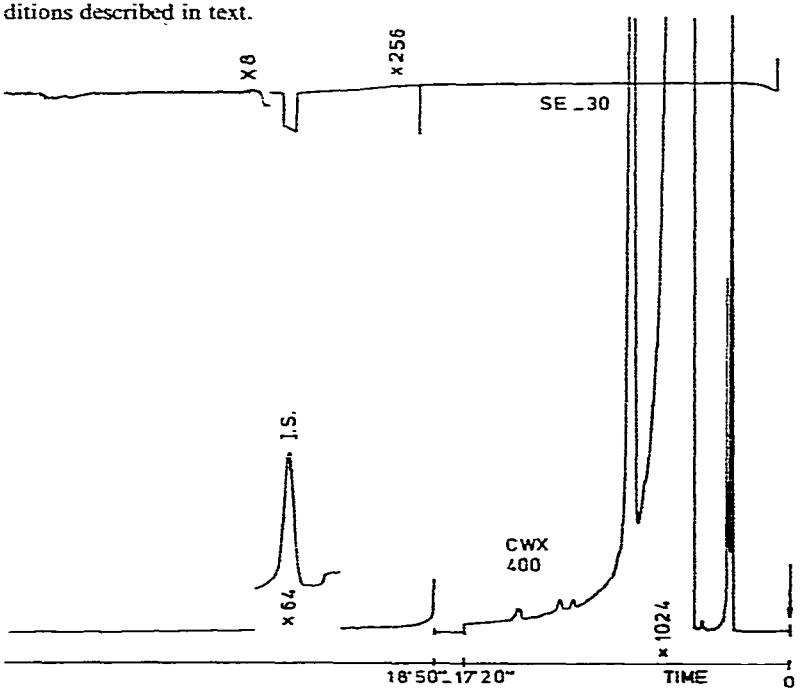

Fig. 4. Gas chromatogram of an extract of grain alcohol on a dual-column system. See Fig. 3 for on-line switching conditions and symbols.

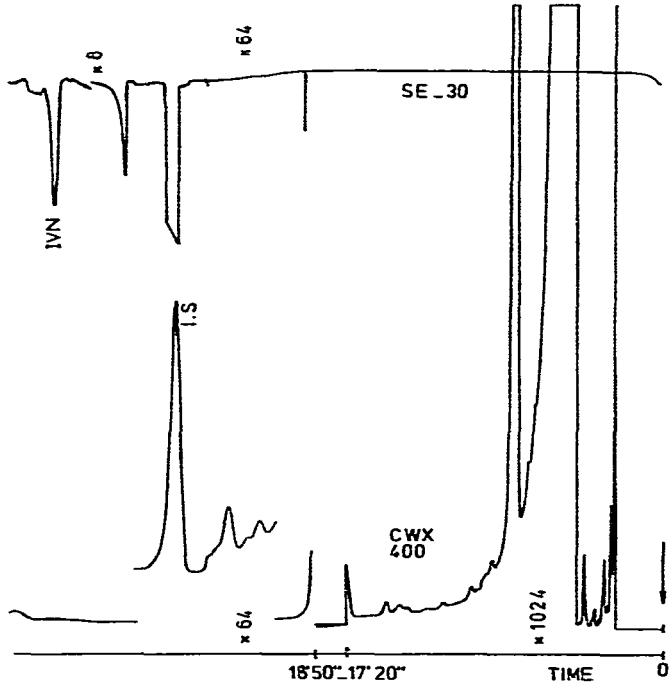

Fig. 5. Gas chromatogram of an extract of Dutch gin on a dual-column system. The gin was manufactured from beet molasses alcohol. See Fig. 3 for conditions and symbols.

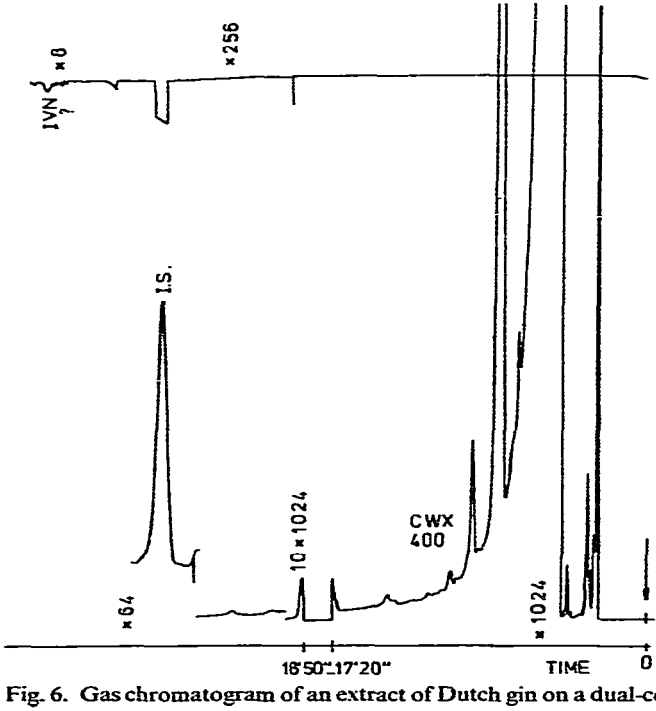

Fig. 6. Gas chromatogram of an extract of Dutch gin on a dual-column system. The gin was manufactured from grain alcohol.

TABLE I

PEAK HEIGHT RATIO OF ISOVALERONITRILE (IVN) AND HEPTANONE-2 (I.S.) IN 10% GRAIN ALCOHOL WITH ADDED IVN

$y = 2.41x - 0.08$.

Added IVN, x (µg/l)	*Peak height ratio,* $y = \frac{IVN}{I.S.}$
0	0.0
0.4	0.86, 0.87
0.8	1.84, 1.86
1.6	3.61, 3.71
3.2	7.62, 7.69
4.8	11.40, 11.64

Attempts to find an internal standard which could be simultaneously transferred to the SE-30 column with the IVN fraction were unsuccessful, but favourable results were obtained with heptanone-2 which has a longer retention on the Carbowax 400 column than the IVN. Table I lists the added quantities, x, of IVN and the corresponding ratios, y, of the peak height of IVN and the internal standard (I.S.). The equation of the regression line was $y = 2.41x - 0.08$ and the correlation coefficient was $r = 0.998$.

After ten analyses of a Dutch gin manufactured from beet molasses alcohol a variance coefficient of 3.6% was calculated. The limit of detection of IVN in Dutch gin (35%) was 0.12 µg/l; that in grain or molasses alcohol (96%) was 0.33 µg/l. A recovery of 61% was obtained.

Table II presents the IVN concentrations in a number of grain alcohols and beet molasses alcohols. A number of Dutch gins sold on the Dutch market were also

TABLE II

ISOVALERONITRILE CONCENTRATION IN GRAIN ALCOHOL AND BEET MOLASSES ALCOHOL

Alcohol sample	*Isovaleronitrile (µg/l)*
Grain alcohol (13 samples)	Not detectable < 0.33
Molasses alcohol	
1	1.2
2	3.4
3	182
4	5.9
5	1.2
6	5.7
7	6.5
8	8.1
9	1.6
10	0.9
11	410
12	16.4
13	11.3

analysed. The results of these analyses are shown in Table III. Samples 1–4 were definitely known to be manufactured exclusively from grain alcohol; samples 5–8 were sold as grain alcohol gins (?) according to the labels. Of the remaining samples the only information available was that Nos. 14 and 15 were manufactured exclusively from molasses alcohol. Many labels implied by depiction of grain stalks that grain alcohol had been used for manufacture of the beverage.

TABLE III

ISOVALERONITRILE CONCENTRATION IN DUTCH GIN (35%)

Sample	*IVN concentration (μg/l)*
1–4*	0.16–0.17
5–6	0.23–0.25
7–9**	0.32–0.35
10–12**	0.43–0.52
13–16***	0.70–0.98
17	2.16
18	6.5
19**	26.6
20	398.5

* Grain alcohol used as raw material.
** Labelled as "grain gin".
*** Beet molasses alcohol used as raw material.

Although no IVN was detected in any of the grain alcohols (Table II) the real grain alcohol gins (35%) did show a small peak on the chromatogram (Fig. 6). The source of this small peak appeared to be the Dutch gin distillate (50%) which is used during manufacturing to flavour the gin. The IVN can probably be traced back to the raw material used in the manufacture of beet molasses alcohol.

Qualitative experiments revealed that relatively large amounts of IVN are present in beet molasses. Attempts to separate the IVN from the beet molasses alcohol through Norit filtration were unsuccessful. The IVN content of the tested beet molasses alcohols varied between 0.9 and 410 μg/l. An investigation carried out by Tressl *et al.*[2] on volatile nitrogen compounds in beet molasses did not mention the presence of IVN.

If a gin (35%) is manufactured from a molasses alcohol containing 0.9 μg/l IVN the gin will still contain 0.33 μg/l IVN which is above the detection limit. It seems justified, for the time being, to classify Dutch gins as gins manufactured from pure grain alcohol when they contain at most 0.15–0.25 μg/l IVN.

REFERENCES

1 J. Strating, W. M. Westra, L. C. Verhagen and F. P. Slotema, *M.B.A.A. Techn. Quart.*, 16 (1979) 176.
2 R. Tressl, R. Jakob, T. Kossa and W. K. Bronn, *Die Branntwein Wirtschaft*, 116 (1976) 117.