

DETERMINATION OF ETHANOL IN CHOCOLATE SHELL PRALINES AND FILLED CHOCOLATES BY CAPILLARY GAS CHROMATOGRAPHY

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The paper describes a simple gas chromatographic method for quantification of ethanol in distillates of chocolate shell pralines and fillings. The samples were prepared in two steps. The first step consisted of ethanol distillation from the product and the second involved capillary gas chromatography of 10% v/v distillate with expected ethanol content between 0.06% and 2.5% w/w. Quantification was carried out using iso-propanol as internal standard. The range of linear method response was 0.05–3.16% w/w of ethanol, which corresponded to products with ethanol content between 0.5 and 31.6% w/w. The detection limit was 0.0158% w/w and the quantification limit was 0.058% w/w of ethanol with the relative standard deviation of 2.5%.

Key words:
method validation, picnometry, quality control

Ever since 1996, the Croatian leading confectioner Kraš d.d. has obtained and maintained the HRN EN ISO 9001 certification. The aim was to enhance relationship with consumers and maintain the supply of top-quality products. Chocolate shell pralines with liqueur fillings for hard-to-please consumers are among the best-known traditional products in Kraš's stock. According to the formula, ethanol content in such products may vary between 0.6 and 4.5% w/w, and in the corresponding raw materials may reach up to 25.0% w/w. Until recently, the ethanol content had been determined by picnometry. A new method for quantification of ethanol has been devised to simplify the analysis and to meet the requirement for quicker and more accurate procedure. In order to assure quality control of products the method was tested on raw materials such as juice made from sour cherry macerate and 96.0% v/v alcohol, fillings made from fondant and the macerate, and on products like chocolate shell pralines with liqueur fillings and filled chocolates.

MATERIALS AND METHODS

The method consisted of two steps. In the first step ethanol was distilled from samples (1) and in the second the distillate was quantified using capillary gas chromatography (2).

Apparatus and working conditions

Distillation was performed in a 1000 ml flask heated with a gas burner and connected through a bulb to a vertically assembled Liebig condenser.

The gas chromatography (GC) was performed using the capillary gas chromatograph ATI UNICAM 610 Series equipped with flame ionisation detector (FID) and capillary column Machery-Nagel (Düren, Germany), OPTIMA-5, 25 m x 0.32 mm i.d. The chromatography was conducted under the following conditions:

- Detector temperature: 220 °C
- Injector: split/splitless, temperature 150 °C
- Carrier gas: He, flow rate 1.2 ml/min
- Injection volume: 1 μ l
- Split ratio: 50:1
- Make up: He, flow rate 0.6 ml/min
- Column temperature programme: 75 °C, isothermal for 4 min., 10 °C/min up to 130 °C and isothermal for 1 min.

Data were collected and the gas chromatography controlled over a PC with 4880 Data Handling System (supplied by the GC manufacturer).

We used absolute ethanol p. a. (Kemika, Zagreb, Croatia) and iso-propanol p. a. (Alkaloid, Skopje, Macedonia) to prepare standards for peak identification and quantification. For peak identification the standards were a 0.395% w/w (0.5% v/v) aqueous solution of absolute ethanol and a 0.395% w/w (0.5% v/v) aqueous solution of iso-propanol. A 0.395% w/w (0.5% v/v) aqueous solution of absolute ethanol and iso-propanol (0.5 ml of absolute ethanol and iso-propanol were added to redistilled water in a 100 ml flask and mixed) served as a standard for calibration and calculation of the relative response factor (RRF). The method was validated using aqueous solutions of ethanol with initial concentration of 7.9% w/w for the standard (10% v/v; 10 ml of absolute ethanol was added in a 100 ml flask and diluted with distilled water). To achieve as accurate concentrations of standards as possible (to avoid possible mistakes in pipetting), we prepared a standard solution for calculation of RRF and a 10% v/v ethanol solution for preparation of standards. The ethanol and the iso-propanol mass were calculated from the volume and density ($m=V\rho$) and instantly weighed and added to distilled water in a 100 ml flask. Similarly, standards required for determination of the accuracy of GC analysis, the method's linear response, detection limit, and quantification were obtained by weighing the initial solution of a 10% v/v ethanol and 0.5 ml of iso-propanol into a 100 ml flask.

Preparation of samples

About 100 g of sample was weighed into a 1000 ml distillation flask and added 500 ml of water with a spoon of tannin as antifoam agent. The flask was attached to the vertical condenser with a bent tube and a sample was distilled to fill a 100 ml flask.

For GC analysis, 5 ml of distillate was diluted with redistilled water in a 50 ml flask and added 0.25 ml of iso-propanol. The ethanol content in 10% v/v distillate solutions ranged between 0.06 and 2.5% w/w, depending on a particular sample (the analyte concentration ratio in 10% distillates was from 10^{-4} – 10^{-2}).

Determination of ethanol

In the everyday routine the standard solution is injected three times to calculate the RRF (Figure 1). Prepared samples are injected twice (Figure 2). The readings of the ethanol percentage expressed as w/w are taken from the report file and the mean value

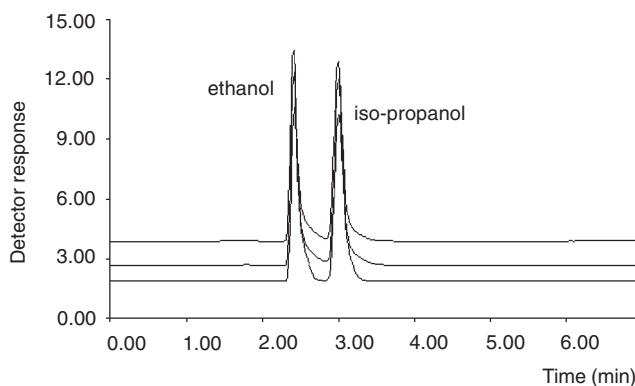


Figure 1 *Representative chromatograms of calibrating standards in the overlay mode*

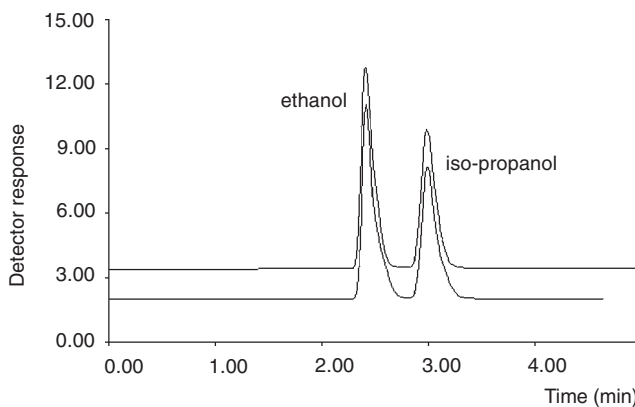


Figure 2 *Chromatogram of a sample praline filling with 5.4% w/w of ethanol in the overlay mode*

is calculated from two injections. The whole procedure for calculation of the ethanol content in 100 g of product (without using data handle system) is given below:

$$\text{RRF} = \frac{\text{peak area (ethanol)} \times \% \text{ iso-propanol (w/w)}}{\text{peak area (iso-propanol)} \times \% \text{ ethanol (w/w)}} \rightarrow \text{standard solution for RRF}$$

$$\text{Ethanol} = \frac{\text{peak area (ethanol)} \times \% \text{ internal standard (w/w)} \times 10^* \times 100^{**}}{\text{peak area (iso-propanol)} \times \text{RRF} \times \text{weight of sample}} \rightarrow \text{sample solution}$$

* Dilution factor

** Refers to 100 g of product

RESULTS AND DISCUSSION

Figures 1 and 2 show that the peaks of ethanol and iso-propanol are sharp, distinguish well, and have no tailing effects. The chromatography of one sample took about 7.5 minutes. The working conditions were established to elute peaks of ethanol and iso-propanol as soon as possible, but also to elute solvent from the column (peak of water is not shown).

We compared the results obtained by gas chromatography, with results obtained by picnometry and they were comparable. The new method, however, produced somewhat closer results to normative values stated in the product formula.

The next step was method validation and we partially followed recommendations found in the Hewlett-Packard publications (3, 4). All standard solutions were made from aqueous solution of 7.9% w/w of ethanol (10% v/v) which served as the initial concentration.

Precision of sample preparation for GC analysis

We tested the repeatability of preparation of 10% distillate solutions for GC analysis, yet we did not include the distillation step because it had been done as a standard procedure for many years and was considered repeatable enough. Table 1 shows the results of the ethanol content in 100 g of four different products from everyday production. The first two products were pralines, the third was a chocolate candy with filling, and the last was a praline filling. The results were obtained from three matrices of 10% distillates for every product and from five injections for every matrix.

Relative standard deviations ranged from 1.22% to 2.40% (see Table 1) and were quite uniform for all tested samples. They met criteria given by the AOAC manual (3) for the peer-verified methods programme with estimated precision data as a function of analyte concentration (3.7% of RSD is allowed with analyte ratio of 10^{-3}).

Table 1 *Repeatability of sample preparation for determination of ethanol in chocolates*

Product	Ethanol % w/w in 100 g of products			$\bar{X}_{mv} \pm SD$	RSD %
	1	2	3		
Chocolate pralines A	3.36	3.29	3.23	3.29 ± 0.06	1.99
Chocolate pralines B	3.92	4.10	3.96	3.99 ± 0.09	2.40
Filled chocolate	1.09	1.06	1.04	1.06 ± 0.02	2.07
Filling for chocolate praline	4.93	5.01	4.89	4.94 ± 0.06	1.22

\bar{X}_{mv} = mean value of three matrices
 SD = standard deviation
 RSD% = relative standard deviation %

Accuracy of GC analysis

The accuracy of the GC analyses was tested to see whether the actual values of ethanol concentrations in standards matched those obtained by the GC analysis (4). We examined 12 standards with different ethanol concentrations, ranging from 0.0158% to 3.16% w/w (0.02–4.0% v/v, respectively). Table 2 shows the results of response to

Table 2 *Accuracy of the gas chromatography analyses*

Ethanol % in standards		Response	Response/true value
v/v	w/w	w/w	%
0.02	0.016	0.010	62.5
0.04	0.032	0.027	84.4
0.06	0.047	0.044	93.6
0.08	0.063	0.059	93.7
0.10	0.079	0.075	94.9
0.2	0.158	0.155	98.1
0.5	0.395	0.388	98.2
1.0	0.790	0.772	97.7
1.5	1.185	1.183	99.8
2.0	1.580	1.586	100.4
3.0	2.370	2.304	97.2
4.0	3.160	3.166	100.2

the true value ratio obtained from six injections for every standard. The tested concentration range corresponded to products with 0.158–31.6% w/w of ethanol. The concentration span was about 80–120% of the expected concentration range).

The results showed that GC analysis was accurate enough within the examined range of ethanol content of 0.0474–3.16% w/w (0.06–4.0% v/v).

Range of linear response

Plots drawn from the accuracy test of the GC analysis showed linearity of the method response (3). Figure 3a shows a response in w/w to the actual values of the ethanol

content. The response was linear in the whole tested concentration range. To show more precisely where the linearity ended, the response results to the true value ratio were plotted against the corresponding content of ethanol on a log scale (Figure 3b) (3). The method proved linear in the concentration range of 0.05–3.16% w/w of ethanol, which corresponds to ethanol content in products with the concentration range 0.5–31.6%.

Detection limits and quantification

Detection limits and quantification of ethanol content were tested with the concentration range of ethanol between 0.0158 and 0.395% w/w using the EURACHEM ap-

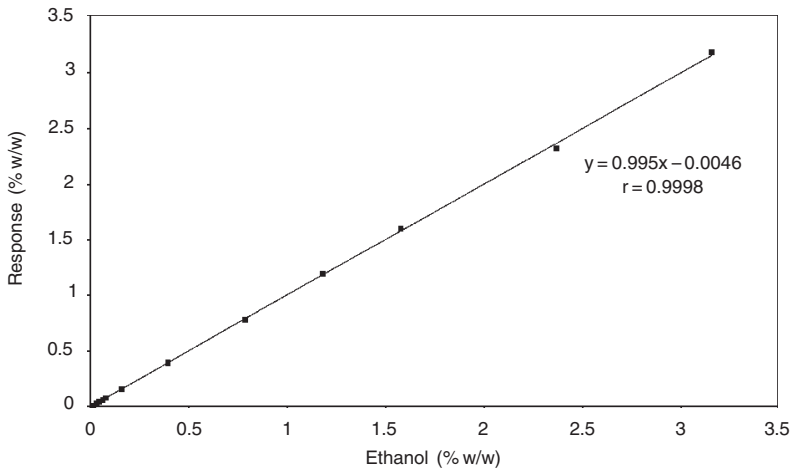


Figure 3a Linearity of the method response

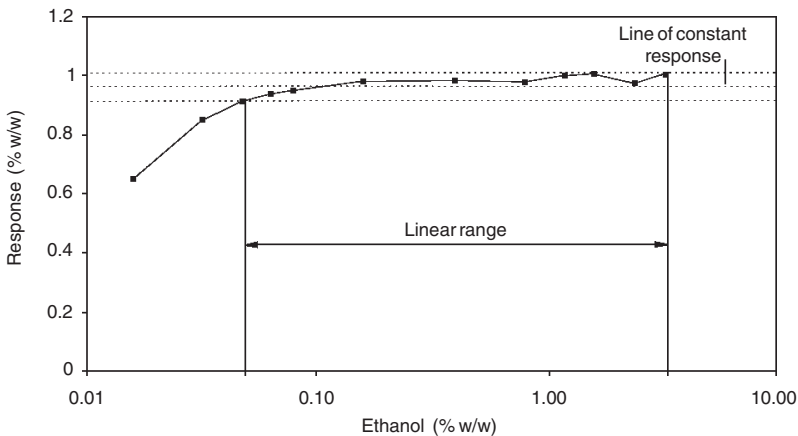


Figure 3b Linearity of the method response

proach (3). Standards with decreasing amounts of ethanol were injected six times. The calculated percentage of RSD was plotted against the ethanol percentage expressed as w/w (Figure 4). The detection limit was found at 0.0158% w/w of ethanol.

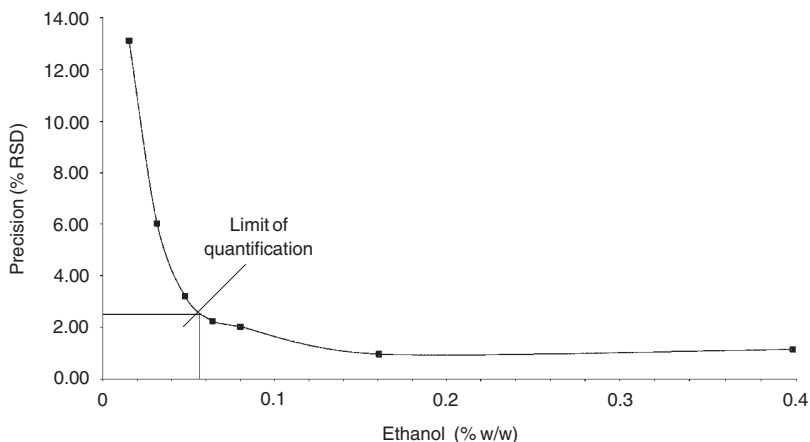


Figure 4 *Limit of quantification*

The quantification limit was read from the plot according to the previously defined required precision of method, which was 2.5% of relative standard deviation. The limit of ethanol quantification was estimated as concentration of 0.058% w/w which corresponded to ethanol content in products of 0.58% w/w. The analyses of products with ethanol content lower than the quantification limit were considered unreliable.

Precision of the GC analysis of ethanol in chocolates

The precision of GC analyses was tested on diluted sample distillates of five different products with different content of ethanol (chocolate with filling, pralines, and cherry juice with alcohol). Every sample was injected five times and the standard deviation and the RSD percentage calculated. Table 3 summarises the repeatability of the

Table 3 *Repeatability of the GC analysis of ethanol in chocolates*

Products	Ethanol % w/w \pm SD	RSD %
Filled chocolate	0.73 \pm 0.01	0.83
Mixed pralines	2.32 \pm 0.02	0.50
Pralines A	3.87 \pm 0.02	0.41
Pralines B	4.31 \pm 0.05	0.85
Sour cherry juice	18.16 \pm 0.05	0.66

Number of injections: 5
 SD = standard deviation
 RSD% = relative standard deviation %

method. The method proved very precise for all ethanol concentrations in tested samples, including sample 3 where the content of ethanol was near the limit of quantification.

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Sažetak

ODREĐIVANJE ETANOLA U ČOKOLADNIM DESERTIMA I U PUNJENOJ ČOKOLADI S POMOĆU KAPILARNE PLINSKE KROMATOGRAFIJE

Predstavljena je plinskokromatografska metoda određivanja sadržaja etanola u čokoladnim desertima i punjenim čokoladama na kapilarnoj koloni. Ponajprije, ova je metoda uvedena kao rutinski postupak u osiguranju kvalitete sirovina i gotovih proizvoda u »Krašu«.

Metoda se provodi u dva koraka. Prvi je korak destilacija proizvoda, a zatim slijedi plinska kromatografija 10% v/v destilata uz očekivani maseni udio etanola između 0,06 i 2,5%, što odgovara masenom udjelu etanola u gotovom proizvodu od 0,6 do 25,0%. Maseni udio etanola određivan je uz izopropanol kao interni standard. Testiranjem pojedinih parametara validacije metode dokazano je da metoda odgovara prethodno postavljenim zahtjevima. Linearnost metode određena je za raspon masenog udjela etanola u gotovom proizvodu od 0,5 do 31,6%. Granica detekcije određena je kod masenog udjela etanola od 0,16% u gotovom proizvodu, a granica kvantifikacije kod 0,6% u gotovom proizvodu uz relativnu standardnu devijaciju od 2,5%.

Pokazalo se da je metoda linearna i precizna za traženi raspon koncentracije etanola te da se može primijeniti za različite proizvode.

Ključne riječi:

čokoladni deserti, kontrola kakvoće, piknometrija, validacija metode

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