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COMBINATION OF CHROMATOGRAPHIC TECHNIQUES FOR ANALYSING THE COMPOSITION OF GEORGIAN ALCOHOLIC BEVERAGES

I. Sh. SHATIRISHVILI
Georgian Agricultural Institute, Tbilisi (U.S.S.R.)

SUMMARY

A combination of chromatographic techniques used for analysing the composition of Georgian wine materials. The determination of the vapour phase composition with three detectors and the determination of amino acids and phenolcarboxylic acids is described.

INTRODUCTION

Wines, wine materials and spirits contain over 1500 compounds[1–4], and more are still being discovered. However, none of the chromatographic approaches used so far provides a complete analysis of the composition of wine materials. Therefore, a combination of chromatographic techniques has been developed and used for the analysis of the composition of Georgian wines. The first step is filtration to remove solid impurities, followed in some instances by filtration on membrane filters, and then chromatography as follows:

Vapour-phase analysis (three modifications)	Highly volatile (mainly, odour-determining) components
Chromadistillation	Components of high and medium volatility, general composition
Chromatomass spectrometry	Identification of compounds
Thin-layer chromatography (TLC)	Sugars, phenolcarboxylic acids, etc.
Ion chromatography	Cations, anions (including sulphites)
High-performance liquid chromatography (HPLC)	Sugars, amino acids, phenolcarboxylic compounds

The above techniques were used in studying Georgian wines and brandy. As a very large amount of information was obtained here we outline only the most interesting of the results obtained, which concern the vapour-phase analysis with the use of selective detectors and the determination of amino and phenolcarboxylic acids by high-performance liquid chromatography.

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EXPERIMENTAL AND RESULTS

Vapour-phase analysis of volatile compounds

The analysis run at 50°C was peculiar in that it employed three detectors simultaneously (a flame ionization, electron-capture and flame photometric detectors) connected in series with a flow-dividing valve after a Carbowax 400 capillary column (40 m × 0.6 mm I.D.) on a Carlo Erba 2300 chromatograph. Ten kinds of Georgian wines were analysed for the content of various components in comparison with a standard. Fig. 1 shows a chromatogram of Gurdzhaani wine obtained with temperature programming.

The wines studied had high contents of ethyl acetate and, in some instances, ethyl propionate (Tsolikauri and Tsitska wines). The diacetyl contents of all the wines were approximately the same. Red wines were found to be higher in dimethyl sulphide[5].

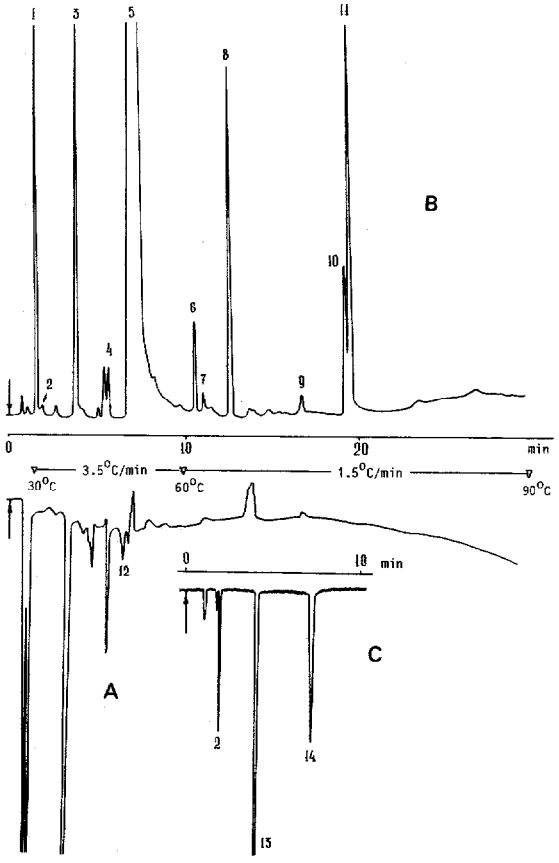

Fig. 1. Chromatograms of the vapour phase of Gurdzhaani Georgian wine on a PEG 400 column. Simultaneous detection with (A) electron-capture, (B) flame ionization and (C) flame photometric detectors. 1 = Acetaldehyde; 2 = dimethyl sulphate; 3 = ethyl acetate; 4 = ethyl propionate; 5 = ethanol; 6 = *n*-propanol; 7 = isoamyl acetate; 8 = 2-methylpropanol; 9 = ethyl caproate; 10 = optically active amyl alcohol; 11 = isoamyl alcohol; 12 = diacetyl; 13 and 14 = unidentified sulphur-containing compounds.

TABLE I

AMINO ACID CONTENT OF GEORGIAN WINES

Substance	*Content (nmol/ml)*	
	Gurdzhaani white wine	*Mukuzani red wine*
Taurine	63.6	60.0
Aspartic acid	63.2	117.6
Threonine	31.8	63.6
Serine	64.2	73.6
Asparagine	72.0	44.0
Glutamic acid	98.8	255.5
Glutamine	78.6	111.2
Proline	2648	8320
Glycine	146.6	276
Alanine	298.2	515.6
Citrulline	5.4	13.2
α-Aminobutyric acid	3.8	3.2
Valine	46.0	97.2
Cystine	8.0	23.2
Methionine	8.4	9.2
Isoleucine	16.0	24.4
Leucine	61.6	58.8
Tyrosine	51.2	60.4
Phenylalanine	41.0	51.2
β-Alanine	–	47.2
γ-Aminoburyic acid	126.0	269.6
Ammonia	87.4	370.8
Ornithine	58.0	159.6
Lysine	88.4	107.6
1-Methylhistidine	1.6	4.0
Histidine	32.8	48.0
Arginine	61.8	27.2

Determination of amino acids

The amino acid composition was determined using a Biotronic Model C 6001 chromatograph equipped with a standard column. A solution of lithium citrate was used as a buffer and ninhydrin as the reagent. The chromatograms were recorded spectrophotometrically at 570 nm (440 nm for proline). The samples of Mukuzani red wine and Gurdzhaani white wine were acidified with concentrated hydrochloric acid and 50 μl were injected into the column.

Calibration and identification were performed using a standard solution. The total analysis time was 2.5 h. The peaks were well resolved and showed the presence of more than 30 amino acids. The main results are presented in Table I. Georgian wines contain a wide variety of amino acids, the high proline content being noteworthy. The proline content can be determined to reveal falsification of natural wines and wine materials[6,7].

Determination of phenolcarboxylic acids

The analysis was carried out by both LTC and HPLC, the latter giving results of most interest. The separation was performed using an Altex chromatograph on

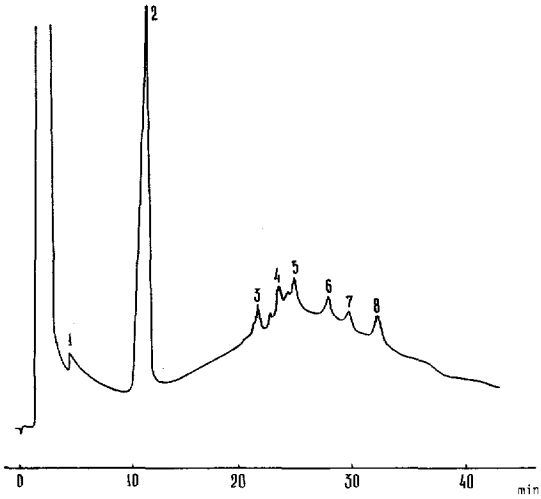

Fig. 2. Chromatogram of a mixture of phenolcarboxylic acids from Georgian brandy. The elution was initially isocratic, then a gradient (see text). 1 = Gallic acid; 2 = vanillic alcohol; 4 = vanillin; 6 = syringic aldehyde; 3, 5, 7, 8 = unidentified components.

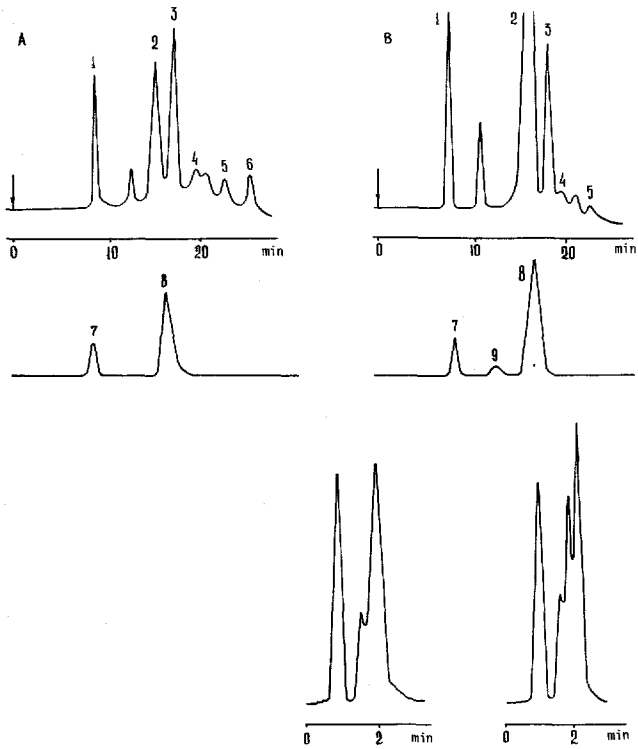

Fig. 3. Separation of organic acids, anions, and alkali metal cations present in (A) Tsinandali and (B) Mukuzani wines by exclusion (top) and ion (middle and bottom) chromatography on an ion chromatograph. 1 = Mixture of the anions of strong acids (Cl^-, SO_4^{2-}, NO_3^-, etc.); 2 = tartaric acid; 3 = maleic acid; 4 = lactamic acid; 5 = formic acid; 6 = propionic acid; 7 = Na^+; 8 = K^+; 9 = NH_4^+. The difference between chromatograms A and B gives the sulphate content.

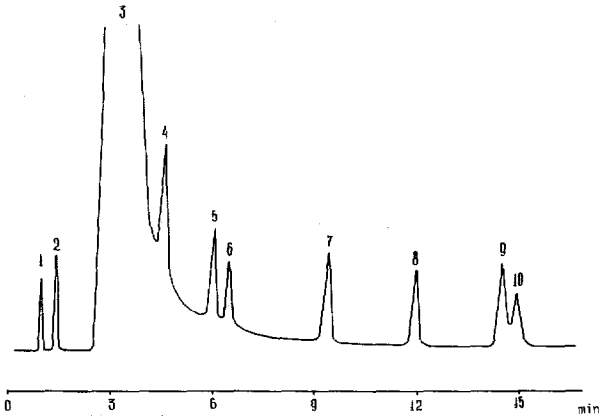

Fig. 4. Separation of a water–alcohol mixture on a Carbopack + 5% PEG 20M temperature-programmed column. 1 = Acetaldehyde; 2 = methanol; 3 = ethanol (96%); 4 = isopropanol; 5 = *n*-propanol; 6 = ethyl acetate; 7 = isobutanol; 8 = *n*-butanol; 9 = optically active amyl alcohol; 10 = isoamyl alcohol.

a Spherisorb S-5-OD column (25 cm × 4 mm I.D.) with a particle size of 5 μm. The procedure followed a complex programme involving initial isocratic elution with mobile phase A for 4 min followed by gradient elution from 10% of A to 100% of B [A = water–acetic acid (980:20) + 0.02 *M* sodium acetate; B = water–methanol–isopropanol–acetic acid (815:140:25:20) + 0.02 *M* sodium acetate]. The flow-rate was 1.5 ml/min and UV detection (280 nm) was used with a detector sensitivity of (A) 0.16 and (B) 0.01.

The model mixture and a sample of Georgian brandy were subjected to chromatography under the same conditions with direct sample injection without concentration (Fig. 2). A comparison of the retention times showed that the brandy contained gallic acid, vanillic alcohol, vanillin and syringic aldehyde; the other components have not yet been identified. The content of vanillic alcohol is high as in French cognacs, rum and whisky[8]. The results obtained indicate a relatively low content of phenolcarboxylic compounds, except vanillic alcohol.

CONCLUSION

Sufficiently complete information on the qualitative and quantitative composition of alcoholic beverages can be obtained only on the basis of an appropriate combination of chromatographic techniques. As most of the compounds are present as admixtures, the development of the procedures for concentating the various groups of compounds is badly needed for the efficient application of different liquid chromatographic techniques, including ion chromatography.

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