

Capillary Electrophoresis in Analytical Control of Wine

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Abstract

Background/Objectives: Quality of grape wines and by-products in connection with the circumscribed resources of natural raw materials receive urgency. The standardize indexes are directed on control of production received from qualitative grape.

Methods: For analytical control of grape wines the system of capillary electrophoresis with the photometric detector, the quartz capillary with an external polyimide coating was used. Results were treated with Multichrome for Windows, version 1.5 and statistical programs for Windows. The new methods with use of instrumentation of capillary electrophoresis have allowed dilating standardized indexes of grape wines and provided control principal organic acids, sulphates, sorbic acid, lysozyme. **Findings:** Appearance and introduction of new directives based on capillary electrophoresis testifies to dynamical development of standard provision of grape wine production quality. Procedures of determination by means of capillary electrophoresis of prioritise amino acids, major cations, inorganic anions, principal organic acids, total nitrogen in grape wines are modified and developed. The results received by means of new methods, have important and allow solve many questions bound to quality. **Results:** It is shown the influence of composition of electrolyte to separation of analysed components. It is estimated sensitivity, detection limits, linearity, and was calculated electrophoretic mobility determined components of grape wines. It is positioned that no more than 5 measurements of samples on one portion of electrolyte in the yielded conditions with the guaranteed error of a procedure. The further exploitation leads to growth of an error of quantitative calculations up to 8%. For determination of complex indexes may be used the temperature destruction of sample of grape wine and further determination by method of capillary electrophoresis.

Keywords: Amino Acids, Electrolyte, Grape, Ions, Organic Acids

1. Introduction

Quality and origin problems of wine production and by-products in connection with the circumscribed resources of natural raw materials receive an urgency¹. It is frequent in production of known marks of wine in Russia use of cheaper raw materials (import stuffs, intermediates and waste), less qualitative food additives and simplified technologies, based on purely economic reasons². Some new methods of processing of raw food materials are far from perfect.

The standardize indexes regulated in state standards currently in force in in Europe and Russia for grape wines, are directed on control of production received from qualitative natural raw materials, and insufficiently adapted for an assessment of possible deflections from technology. As a rule, the grape wine subjected to test, contains the regulated quantity of alcohol and sugar, has colour, characteristic for the yielded name, meets the content requirements of acids, extract, but has no taste and odour typical for varietal wine. Only introduction of new technologies of the analysis - capillary electrophore-

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sis and hybrid methods of the analysis - in winemaking branch will allow confining presence of low quality wine in the market of Europe and Russia³.

The falsified wines usually represent an artificial mixture of ethanol, sugar, citric acid, rarely some tartaric acid, various concentrates and extracts of synthetic and phyto-genesis origin, other ingredients and completely correspond to requirements of state standards currently in force in Russia on physical and chemical properties and criteria of safety. However such wines, possessing the low flavouring characteristic, can become and the cause of venerating because of presence of the materials possessing own toxicity or components, enhancing toxic action of ethanol and allergic responses. The yielded circumstances promoted research of new quality monitoring based on chromatographic methods.

The purpose of this work is a substantiation of expediency of methodical research on the basis of capillary electrophoresis for analytical control of wine.

2. Experimental Part

2.1 Material and Chemicals

System of capillary electrophoresis of a series CAPEL (Russia) with the photometric detector (254 nm); the quartz capillar with an external polyimide coating (bore diameter of $75 \cdot 10^{-6}$ m, effective length of 0.5 m; aqueous thermostatic control) was used.

Results were treated with Multichrome for Windows, version 1.5 (Open Company "Ampersand", Moscow).

Standard samples of solutions of cations of potassium MSO 0019:1998, ammonium MSO 0017:1998, sodium MSO 0018:1998, magnesium MSO 0085:1999, calcium MSO 0020:1998; arginine (Sigma), threonine (Sigma), proline (Sigma), tartaric acid (Vecton), benzimidazole (Sigma), 18-crown-6 (Sigma), standard samples of solutions of anions of chloride-ion GSO 8747-2006, nitrite-ion GSO 7792-2000, nitrate-ion GSO 7793-2000, sulphate-ion of GSO 7683-99, potassium dichromate (Vecton), HCl (Vecton), NaOH (Vecton), $\text{Na}_2\text{B}_4\text{O}_7$ (Vecton) were used.

2.2 Results and Discussions

Contents of free amino acids - arginine, proline, threonine, cations - potassium, sodium, magnesium, calcium, polybasic organic acids are one of known elements of

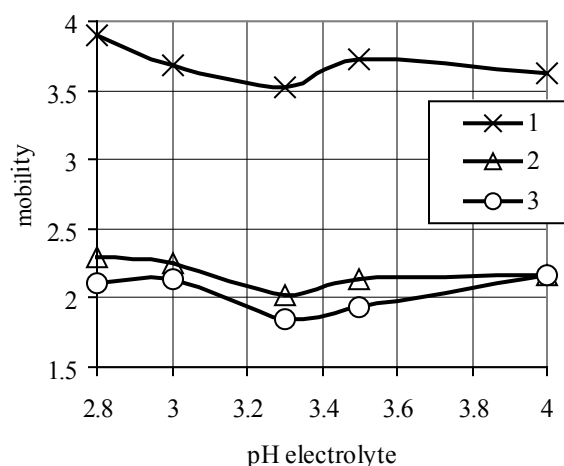
grape wine authenticity. The basic difficulty of electrophoretic determination of these components is absence in their molecules of chromophorous groups that leads to low sensitivity of detection⁴. To solve a problem derivatization suitable reagents (except cations of alkali metals) can be applied, however it is necessary to take into account a complexity of substances in grape wine, capable to proceed concurrent reactions, and to deform results of the analysis accordingly⁵. Nevertheless, rather high maintenance of analysed components in wine allows using this advantage to maintain higher accuracy and reliability of the total analysis. This circumstance is often used by various researchers at building of procedures of the analysis of wine by means of capillary electrophoresis.

The composition of electrolyte providing separation and quantitative determination component of wine is developed for the analysis arginine, proline, threonine without sample preparation, in the conditions of indirect detecting. From the sum of amino acids of grape wine ($800\text{-}3500 \text{ mg/dm}^3$) a proline portion is up to 65%, arginine - up to 15% and threonine - up to 10%. These amino acids take direct part in formation of completeness of taste and biological value of wine⁶. For maintenance of the analysis two components are used as a part of aqueous electrolyte: A phosphoric acid providing an electroosmotic flow, and the benzimidazole creating optical density for realisation of detecting. Optimisation of separation of arginine, threonine, proline is received at use of aqueous electrolyte of following composition: 0.25% of a phosphoric acid, with an additive of 0.26% of solution of benzimidazole, in the ratio 2:0.1 (v/v), pH - 3.5. Offered composition of electrolyte provides a separation from a sample template arginine which pH value practically has no influence. Influence of pH on threonine and proline separation was significant to electrophoretic mobility of these components, as testifies Figure 1. It is shown that reduction of pH led to decreasing of time of the analysis, and the increasing pH did not allow separating analysed amino acids.

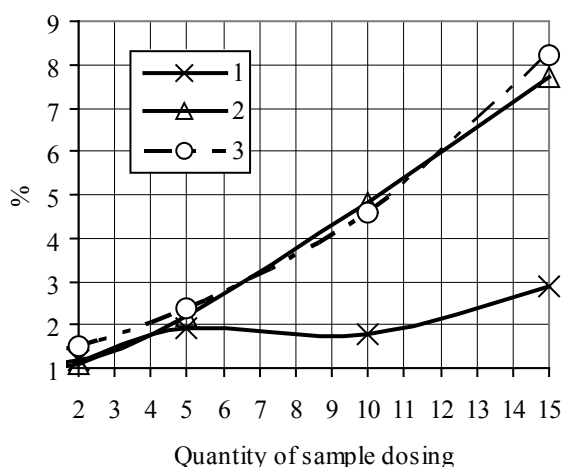
Sensitivity quotients are determined for the yielded procedure of execution of the analysis: proline - 1.0, threonine - 9.0; arginine - 1.7; detection limits - 0.5; 1.0; 3.0 mg/dm^3 accordingly. Linearity was unchanged up to 1000 mg/dm^3 inclusive. Electrophoretic mobility of amino acids ($\times 10^{-4} \text{ sm}^2\text{V}^{-1}\text{s}^{-1}$) was: proline - 3.1, threonine - 2.7, arginine - 2.5.

Voltage choice was guided by value of maximally admissible current and duration of the analysis. It is

positioned that it is possible to execute no more than 5 measurements of samples on one portion of electrolyte in the yielded conditions with the guaranteed error of a procedure. It is proved that the further exploitation characterised by stability of retention times of amino acids, leads to growth of an error of quantitative calculations invoked by change of composition of electrolyte up to 8%.



(a)



(b)

Figure 1. (a) Change of mobility of amino acids depending on pH of electrolyte. (b) Error from quantity of sample dosing.

Control of content of tartaric, malic, succinic, citric, lactic acids in wine matters for quality evaluation and technology, in particular for identification wine residue⁷,

as one of indexes of authenticity criteria of grape juice and wine⁸. State standard of Russia 52841-2007 «Wine production. Determination of organic acids by a method of capillary electrophoresis» was developed by experts of the State Scientific Organization North Caucasian Regional Research Institute of Horticulture and Viticulture (SSO NCRIH&V)⁹. In realisation of method an indirect photometric detecting has applied, time of the analysis is no more than 15 minutes.

Research was made in SSO NCRIH&V to modify and implement in winemaking firms practice a procedure of capillary electrophoresis for determination of percentage by weight of potassium, sodium, magnesium, calcium in the wines¹⁰ which presence directly influences stability of winemaking production and forms flavouring characteristics. It is known that technological treatments of wine production have, as a rule, a selective orientation and knowledge of the causes invoking crystal pacifications of grape wines allows to optimise methods and to control duration of technological treatments.

For realisation of a procedure of determination of cations electrolyte water solution is used: benzimidazole, 18-kraun-6, tartaric acid. Quality of the analysis estimated on dependence of mobility of cations on concentration composition of electrolyte: benzimidazole 10-30 mg/dm³; 18-kraun-6 – 5-15 mg/dm³ and tartaric acid - 12-35 mg/dm³, admixed in the ratio 3:2:1 at positive voltage 16 kV shown in Figure 2(a), (b).

Further for determination of cations in sample of wine electrolyte was used, provided express and selectivity of the analysis. By additional experiments it was established that in such conditions of the analysis it is possible to execute no more than 10 measurements of sample on one portion of electrolyte with the guaranteed error of a procedure. The further operation characterised by stability of migration time of cations, leads to growth of an error of quantitative calculations up to 3-5 % due to change of composition of electrolyte, Figure 2(b).

Approximate migration time in minutes of cations: Ammonium - 4, potassium - 4.8, sodium - 5.5, magnesium - 7.0, calcium - 8.0, linearity of the response of the detector was stable up to concentration of 100 mg/dm³. Correctness and reproducibility of results of determination of investigated cations of metals (n = 5) has compounded from 1.5 to 3.5.

The great influence on quality wine renders the content of ammonium which occurs as a result of natural destructive processes. For wine makers such integrated

index as the content of the total nitrogen¹¹ is not less important also. At determination of the total nitrogen there are the certain difficulties, the developed reception allows to simplify this procedure. Preliminarily carry out under the influence of temperature destruction of sample of wine by an additive in it of a phosphoric acid and hydrogen dioxide. The ion of the ammonium which has combine with phosphoric acid determine by method of capillary electrophoresis. The received percentage by weight recalculate for the total nitrogen.

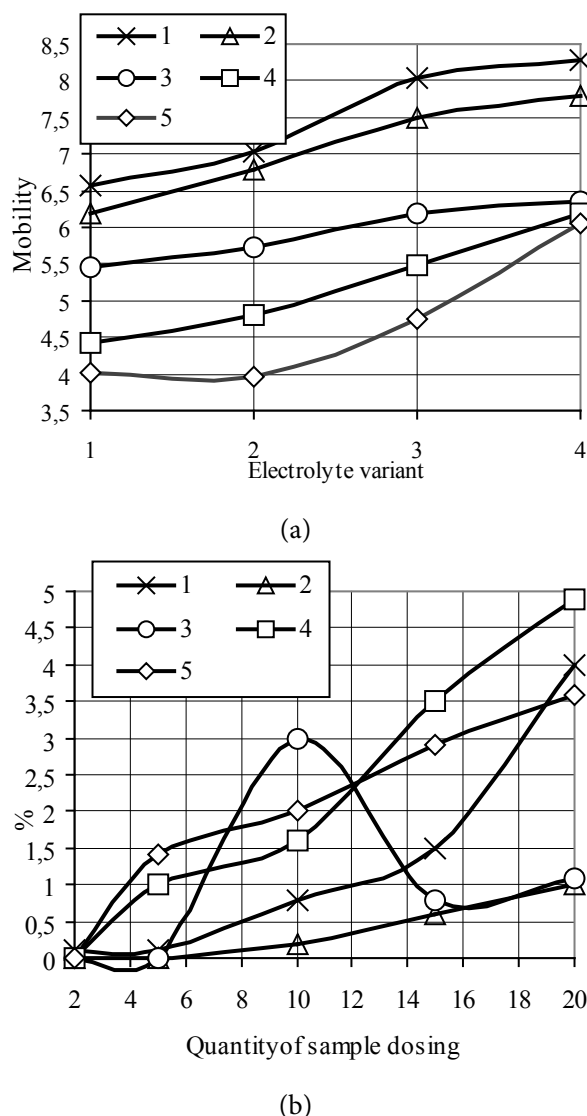


Figure 2. (a) Changes of mobility of cations depending on an electrolyte variant. (b) Error from quantity of sample dosing.

For realization of a procedure of determination of total nitrogen (as ion ammonium) electrolyte water solution is used: benzimidazole, 18-kraun-6, tartaric acid

and sodium sulphate. Quality of the analysis estimated on dependence of mobility of ion on concentration composition of electrolyte: benzimidazole - 2.5 mg/cm³; 18-kraunefir-6 - 2.2 mg/cm³, tartaric acid - 3 mg/cm³, and sodium sulphate - 1 mg/cm³, admixed in the ratio 3:2:1:0.1 at positive voltage 16 kV. Electrophoregram sample of wine of without destruction is shown in Figure 3 after destruction in Figure 4.

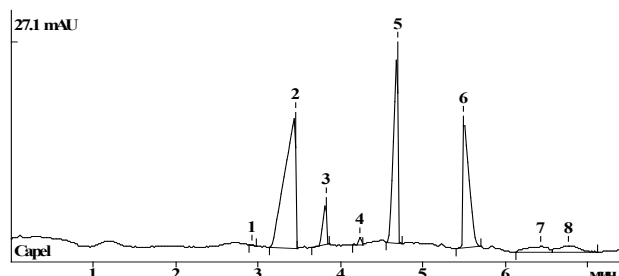


Figure 3. Electrophoregram of wine without destruction: 1 - ammonium, concentration 0.3 mg/dm³, dilution of sample 5.

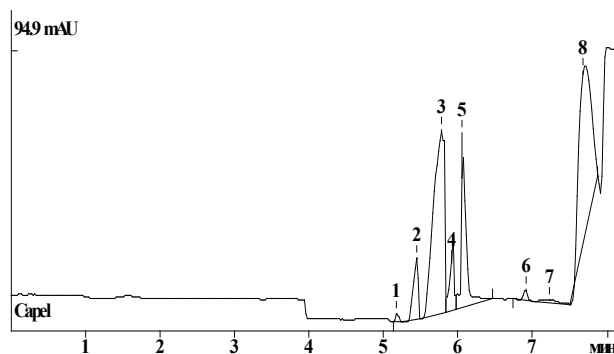


Figure 4. Electrophoregram of wine after destruction: 1 - ammonium, concentration 238 mg/dm³, dilution of sample 10.

The obtained data will be co-ordinate with other means of the analysis and testifies to possibility of quantitative determination of the total nitrogen in wine, in the form of an ammonium ion according to offered procedure. Difference of time migration of an ammonium ion on figure 3 and 4 stipulate for value of pH of sample of wine after destruction.

It is necessary to notice that one of determining characteristics of various types of grape wines is presence of inorganic anions - chlorides and sulphates, and concentration of sulphate, as a rule, gradually raises at the expense of sulphite-ion oxidation, and method for determination anions it is known^{12,13}. It was established that soil tillage enhances the amount of aromatic components such as esters, carboxylic acids, alcohols and phenols; also

new treatment of grape¹⁴. A sulphite-ion source is the sulphur dioxide used as preservative in winemaking in dosages up to 200 mg/dm³,¹⁵. Electrophoretic procedure with use of water solution of dichromate of potassium and hexamethylene diamine¹⁶ is offered for measurement of concentration of chloride, sulphate, nitrite, nitrate. Potassium dichromate provided optical density and ionic strength of leading electrolyte in the conditions of indirect detection; hexamethylene diamine is applied to achievement of demanded pH value and dichromate transfer to chromate. Optimum separation of a model mixture is received with use of leading electrolyte containing 5 mM/dm³ of each component. Optimum negative voltage 8 kV was enough for separation of the model mixture, characterised by mobility of anions. Selection of voltage for definition performance at the positioned composition of leading electrolyte allowed to control duration of analysis and to provide necessary quality of separation at the same time.

Rough time of migration (minutes) of anions for the optimised composition of leading electrolyte and negative voltage 8 kV: chloride - 8, nitrate - 8.6, nitrite - 9.0, sulphate - 9.4.

Under condition of application of acid destruction of sample of wine possibility of determination of total phosphorus in the form of a phosphate-ion in the conditions of the analysis of electrolyte containing 5 mM/dm³ of potassium dichromate and hexamethylene diamine is positioned¹⁷. Influence of soil conditions, especially soil salinity, sulphur dioxide application lead to ambiguous accumulation of studied anions in grapes and wine. The knowledge of the initial amount of investigated anions in wines will allow to operatively variate technological process and to supervise quality of production. Detection of excess quantities of nitrates and nitrites can form the establishment for a statement of disharmony of production to safety requirements.

3. Conclusion

Appearance and introduction of new state standards of Russia based on capillary electrophoresis testifies to dynamical development of standard provision of wine production quality in Russia. Procedures of determination by means of capillary electrophoresis of amino acids, cations, anions, organic acids, total nitrogen in grape wines are modified and developed. The results received by means of new methods, have important applied value and

allow solve many questions bound to quality and safety of wine production and by-products. The developed analysis with use of procedures of capillary electrophoresis will allow the enterprises to operate quality and to form certificates of production, thus, protecting it in the consumer market.

The state standards of Russia implanted in conditions of production and perspective procedures with use of instrumentation of capillary electrophoresis have allowed to dilate standardized indexes of wine production and thereby provided preconditions for the general upgrading of wine production quality. Analog state standards worked by Instituto dos Vinhos do Douro e do Porto: MIVDP 64 Edição nº 03: Vinho e Vinho licoroso. Ácidos orgânicos (ácido succínico e ácido láctico) por electroforese capilar; Sulfatos por electroforese capilar. It is international directives: OIV-MA-AS313-18 Sorbic acid (capillary electrophoresis); OIV-MA-AS313-19 Determination of the principal organic acids of wines and sulphates by capillary electrophoresis; OIV-MA-AS315-24 Determination of lysozyme in wine using high-performance capillary electrophoresis.

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