VERSITA 10.2478/v10310-012-0012-7
Ovidius University Annals of Chemistry Volume 23, Number 1, pp. 77-81, 2012

Simultaneous determination of artificial sweeteners in possible counterfeited wines, using high performance liquid chromatography with DAD detection

Elisabeta-Irina GEANA*, Andreea-Maria IORDACHE and Roxana Elena IONETE

Research and Development Department, National R&D Institute of Cryogenics and Isotopic Technologies – ICSI Rm. Valcea, 4 Uzinei St., 240050 Rm. Valcea, Romania

Abstract Increased use of food, beverages or drugs containing synthetic sweeteners presents a real danger to health, which is why the EU Member States had to establish a system of regular surveys to monitor sweetener consumption. In case of wine industry, according to law no. 244/2002, Romania prohibits the addition of synthetic sweeteners in wine in order to obtain sweet wine. The official method for detection of adulterated sweet wines with synthetic sweeteners is TLC-Thin Layer Chromatography. However, quantitative methods of analysis are needed to measure levels of sweeteners in different food matrices and high performance liquid chromatography has proved to be a powerful tool for quantitative analysis of compounds at traces levels. In this paper, a high performance liquid chromatographic (HPLC) method for the simultaneous separation and determination of three of the most popular artificial sweeteners (acesulfame potassium, saccharine and aspartame) in a single injection was developed. The described method is rapid, accurate and highly sensitive. Detection limit were 4 mg/L for acesulfame K, 1 mg/L for saccharine and 9 mg/L for aspartame respectively. The precision of the method was about 2% and recovery ranged between 92.6% and 103.3%. There were analyzed commercial wine samples, in order to detect possible counterfeits-sweet and medium sweet wines. Therefore, of the 20 analyzed wine samples, only two samples consisting in wine sweet table, were counterfeited by adding saccharin.

Keywords: artificial sweeteners, adulterated wine, HPLC

1. Introduction

The artificial sweeteners are additional substances, which are used to sweet taste addition to food products or as table sweeteners. The most commonly used artificial sweeteners in soft drinks are aspartame, acesulfame K and saccharin (see **Fig. 1**), each of which may be used individually or blended with sugars or one or more of the others.

Saccharin is salt of anhydride of sulfaminobenzoic acid. It is the oldest and the most common used sweetener in the world. The sweetness of saccharine is 400–550 times higher than sucrose but it has long-time bitter metallic aftertaste. It is used in the form of Na or Ca salts, well soluble in the water [1].

Acesulfame K is potassium salt of 6-methyl-1,2,3-oxathiazinone-2,2-dioxide. In comparison with 3–5% solution of sucrose it is 150–200 times sweeter, whereupon threshold of sensitivity for sweetness is 0.08–0.12 mmol/l, for bitterness 3–7 mmol/l.

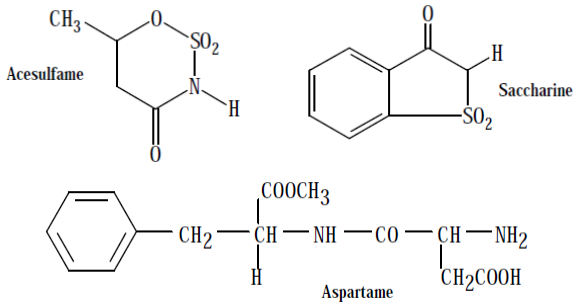


Fig. 1 The most commonly used artificial sweeteners

The sweetener is not metabolized by the human body and thus contributes no energy to the diet. Acesulfame K contains two amide and one ester bond that they can hydrolyze at higher temperature mainly in acid conditions [1].

Aspartame is L-aspartyl-L-phenylalanine methyl ester. Its sweetness is 180 times higher than sucrose. The taste of aspartame is similar to sucrose and it

ISSN-1223-7221

has the ability to strengthen some aromatic substances. Aspartame is unstable at higher temperatures as well as in aqueous medium. Eventually it is decomposed [1].

The use of artificial sweeteners is regulated in most countries, but in wine industry their use is prohibited. Medium sweet and sweet wines are obtained following a series of technological processes. Deviation from these processes and use of various practices contrary to the laws in force is considered an offense. In Romania, according the law no. 244/2002, the addition of synthetic sweeteners in wine in order to obtain sweet wine, is prohibited.

A variety of methods such as UV spectroscopy [2], capillary electrophoresis [3], high performance liquid chromatography [4, 5] and ion chromatography [6] are used for the determination of synthetic sweeteners in foods, beverages and dietary.

This application note presents a fast and robust liquid chromatography method to test widely used artificial sweeteners such as acesulfame potassium, saccharine and aspartame in possible counterfeited wines.

2. Experimental

2.1. Reagents

Reference compounds (acesulphame K, aspartame and saccharin) were purchased from Sigma-Aldrich. All solid standards were of ≥ 98 % purity. Water was purified using a Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Potassium dihydrogen phosphate, acetonitrile and phosphoric acid were obtained from Merck (Darmstadt, Germany).

Stock solutions were prepared separately by dissolving reference compounds in deionised water. Working standard solutions of aspartame, acesulphame K, and sodium saccharin were prepared by mixing and diluting aliquots of stock solutions.

2.2. Samples

A set of 20 commercial sweet and medium sweet wines were sampled from the market. All samples consisted of table wine, bottled in Polyethylene terephthalate (PET) containers, from which 10 were sweet wines and 10 medium sweet wines.

2.3. Sample preparation

Samples of wines were degassed for 10 minutes in an ultrasonic bath before directly injection in chromatographic system.

2.4. HPLC conditions

Chromatographic analysis was carried out with a Thermo Finnigan Surveyor Plus chromatograph equipped with a diode array detector at 220 nm, Surveyor autosampler, Surveyor LC Pump (Quaternary gradient). Data analysis was done using the Chrome Quest Chromatography Workstation. The analytical column is Aquasil C18, 250 ×4.6 mm, with the sorbent particle size of 5 μm. The mobile phase, mixed of phosphate buffer (2.72 g potassium phosphate monobasic adjusted at pH = 4.3 with orthophosphoric acid) and acetonitrile 90:10 (v/v) has flown through the system at the rate of 1 ml/min. Samples were injected onto analytical column in 10μL volume. Total running time was 30 minutes. All analyses were carried out at ambient temperature.

Quantification of each synthetic sweetener was performed by measuring peak areas at the corresponding retention time and comparing them with the relevant calibration curve.

Confirmation of the analyte in the sample was made using a diode detector. Peaks were confirmed by comparing their spectra with those of the aqueous solutions of the standards.

3. Results and discussions

The described analytical method effectively separates the artificial sweeteners of all analysed samples and standards. To determine retention times for each analyte, a sample of each was runed making it easier to analyze the mixed standards. The determined retention times were approximately 5.26 min for acesulfame K, 9.52 min for saccharine and 20.38 min for aspartame. A chromatogram from a mixed standard solution is shown in **Fig. 2**, containing three peaks corresponding to the three analytes.

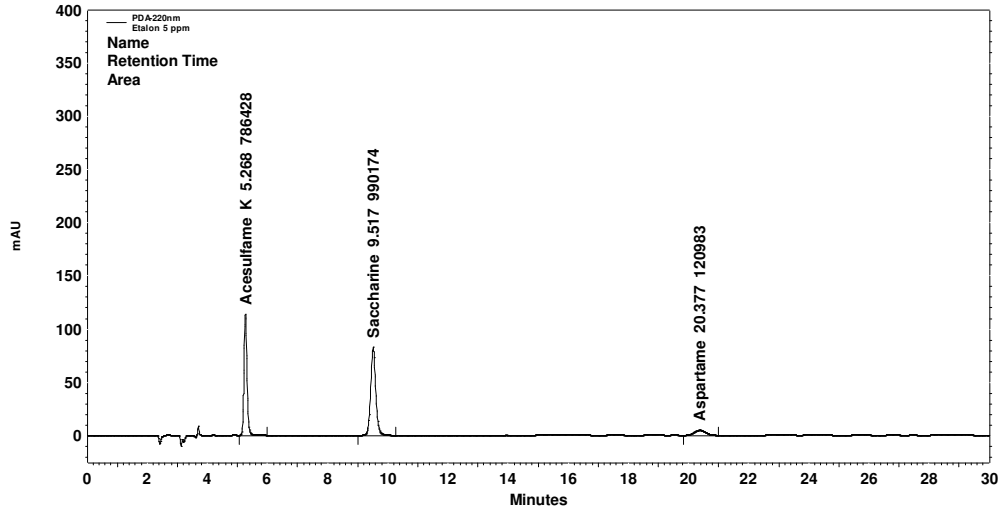


Fig. 2 The chromatograms of standard solutions

Limits of detection (LOD) were estimated with concentrations giving a signal-to-noise ratio of 3/1. The limit of quantification (LOQ) is defined as three time the LOD value. Linearity measurement was based on seven concentration points with three replicates of standard solution for each concentration level.

The precision of our method was evaluated as analytical repeatability based on five replicates of standard solutions. Precision is expressed as the relative standard deviation (RSD) of the replicates.

Recovery was determined by spiking selected samples of each matrix with standards at three concentrations level (10. 25 and 50 mg/L).

The good linearities between the sweetener concentration and peak area responses were achieved over the range from 1 to 100.0 mg/L with a correlation coefficient varying from 0.9966 to 0.9989. Detection limits of acesulfame potassium, saccharine and aspartame were 4, 1 and 9 mg/L respectively. The recovery rates of spiked samples at three level 10, 25 and 50 mg/L respectively, ranged from 92.6% to 103.3%.

Method validation data are shown in **Table 1.**

Table 1. Method validation data

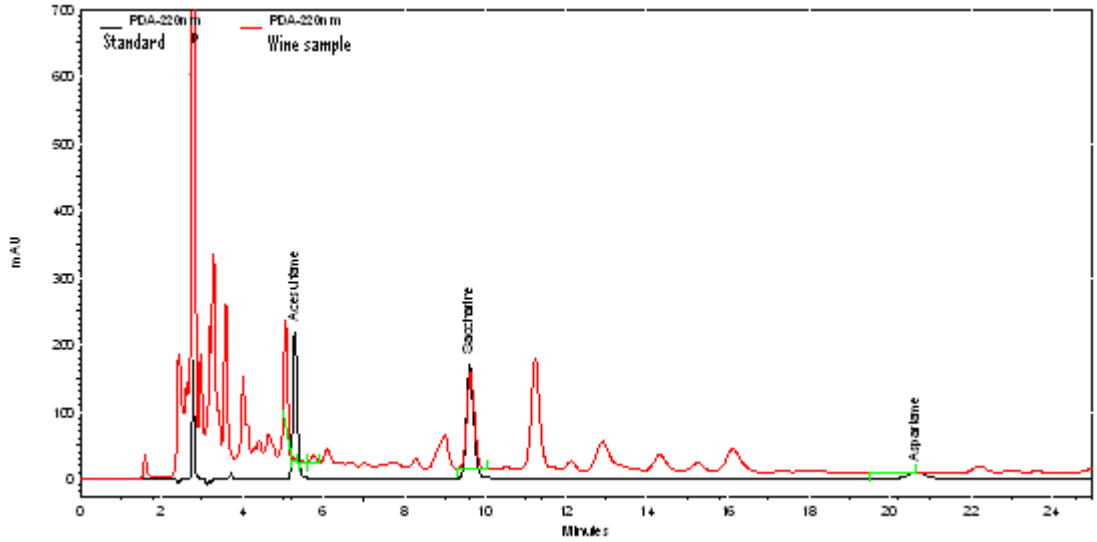
Parameter		**Name of artificial sweetener**		
		Acesulfame K	***Saccharine***	***Aspartame***
Linearity range (mg/L)		1-100	1-100	1-100
Correlation coefficient, r^2 (for calibration curves)		0.9978	0.9966	0.9989
LOD (mg/L)		4	1	9
LOQ (mg/L)		12	3	27
Precision, RSD%		2.1	2.1	1.9
Recovery %	10 mg/L	98.7	95.3	92.6
	25 mg/L	94.5	103.0	97.4
	50 mg/L	103.3	98.4	100.1

Application with real wines samples

By overlaying the chromatograms of the standards and the real samples, one can easily find out which kind of sweeteners are used in specific samples. **Fig. 3** shows the chromatogram of counterfeited wine sample with saccharine (A) and uncontaminated wine sample (B). In two sweet table wine used in this study (10% of the studied wines) was identified and quantified saccharin at 1.9 mg/L and respectively 9.4 mg/L. In the remaining samples (80% of the studied wines) no sweeteners were identified.

The presence of saccharine in the contaminated samples was confirmed by a diode array detector. Peaks were confirmed by comparing their spectra with those of the aqueous solutions of the standards (**Fig. 4**).

A



B

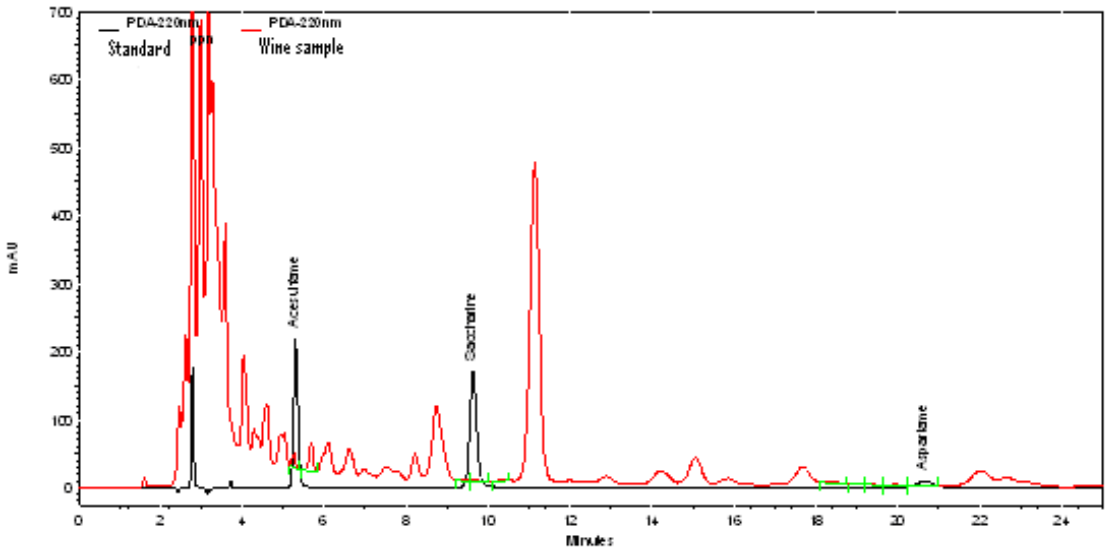


Fig. 3 Overlap of the chromatogram of standards with contaminated sample with saccharine (A) and uncontaminated sample (B)

A

B

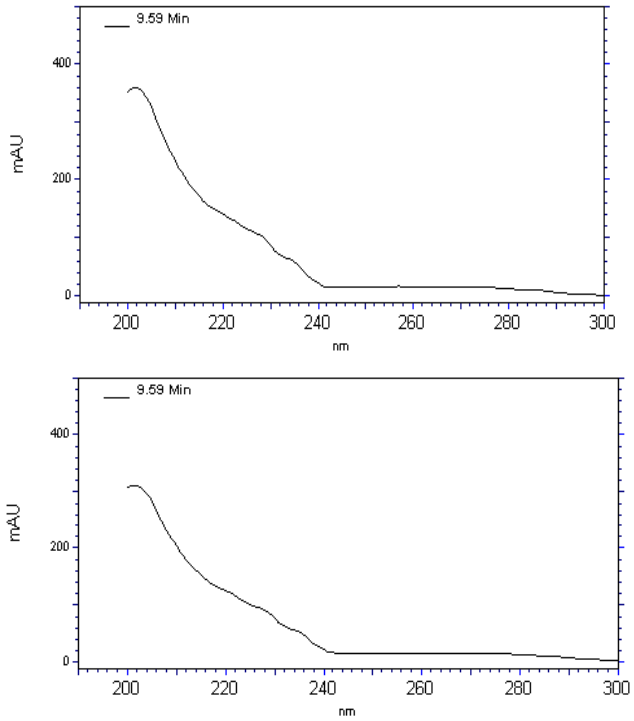


Fig. 4 UV-VIS spectra of aqueous solution of saccharine (A) and counterfeited wine sample with saccharine (B)

In order to eliminate possible interferences due to the matrix, in a future work, we intend to choose a selective extraction procedure for artificial sweeteners from wine, followed by HPLC analysis.

4. Conclusions

The proposed method is rapid, accurate, highly sensitive and suitable for the quality control of low concentration of the synthetic sweeteners, which are illegally added to wines and other beverage. Limits of quantification, precision, and recovery were satisfactory.

It can be applied for detection and quantification of sweeteners from various types of beverages.

It is still too early to say whether, or when, the new test method discussed here will become useable in combating consumption fraud.

5. References

* E-mail address: irina.geana@icsi.ro

1. J. Lehkoživová, J. Karovicová, Z. Kohajdová, M. Suhaj and I. Simonová, Zywnosc. Nauka. Technologia. Jakosc, **3** (48), 76 – 85 (2006).
2. M.A Cantarelli, Pellerano R.G, Marchevsky E.J and Camina J.M., Food Chem. **115**, 1128-1132 (2009).
3. M. Horie, F. Ishikawa, Oishi M, Shindo T, Yasui A and Ito K., J Chromatogr A **1154**, 423-428 (2007).
4. Q. Chen, Mou S, Liu K, Yang Z and Ni Z., J Chromatogr A **771**, 135-143 (1997).
5. Z Huang, Ma J, Chen B, Zhang Y and Yao S., Anal Chim Acta **555**, 233-237 (2006).
6. Y. Zhu, Guo Y, Ye M. And James F.S., J Chromatogr A **1085,** 143-146 (2005).

Submitted: January 22th 2012
Accepted in revised form: April 3rd 2012