GC-MS Determination of Volatile Compounds in Wine Using Needle Concentrator INCAT

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Abstract— *There are several methods for determining of volatile organic compounds in wine. All these sample preparation methods present several disadvantages, such as excessive cost, volume of sample and time, the possible generation of artefacts, etc. Previously developed simple method of solventless extraction of volatile organic compounds from liquid samples has been applied to wines samples. Inside needle capillary adsorption trap device (INCAT) with Carboxen 1000 as a sorbent material and wet alumina as a source of desorptive water vapour flow in a closed GC analytical system is presented. In relations to other extraction techniques used in wine analysis like liquid-liquid and solid-phase extraction, purge-and-trap, and solid-phase microextraction, the INCAT technique offers some advantages because it does not require solvent or any sample treatment, is fast, inexpensive, requires low sample volumes. The analytical characteristics of developed device and of compared purge-and-trap device for analysed samples are similar; the limits of detection as well as quantification are approx. 1 ng/l. The main advantage of INCAT device lies mainly in substantially lower price of analysis and the possibility of sampling directly in the field.*

Keywords— GC-MS, needle concentrator INCAT, wine

I. INTRODUCTION

Wines contain over 800 different volatile organic compounds (VOCs) belonging to different chemical families such as alcohols, ketones, aldehydes, esters, lactones, etc. [1-3]. Among major compounds beside ethanol belong ethyl acetate and higher alcohols. Ethyl acetate is the most abundant ester in wines and is produced by the yeast during the alcoholic fermentation and by the acetic bacteria metabolism. High amounts of ethyl acetate can be considered to be a symptom of wine spoil. It is known that the content in higher alcohols is a factor conditioning wine quality. Amounts higher than 400 – 500 mg/l imply defects in the aroma [1]. Wine quality is significantly influenced by flavor compounds from different chemical families in different concentrations, too. Such compounds come from grapes, fermentation processes and wine ageing. However, both qualitatively and quantitatively, fermentation compounds are the main group, especially esters, which play an important role in white wine aroma [2, 4]. The main technique to analyze these wine components is gas chromatography (GC). The quantification of minor compounds needs a prior concentration step, while major compounds can be analyzed by direct injection of wine sample.

Classical analytical methods used for gas chromatographic analysis of wine volatile compounds such as liquid-liquid extraction [5], purge and trap [6], solid-phase extraction [7, 8], etc., have at least one of the following disadvantages: taking a long time, laboriousness, possibility of contamination or loss, artifact formation, use of environmentally hazardous solvents, difficulties in automation, etc. [4]. Over the last two decades solid-phase microextraction (SPME) has become a powerful technique, commonly employed for the analysis of aroma compounds in wine [3, 9-16]. SPME presents many advantages over traditional analytical methods by combining sampling, preconcentration, and the transfer of the analytes into a standard gas chromatograph. The sensitivity of SPME coatings, such as polydimethylsiloxane and divinylbenzene (PDMS/DVB) and Carboxen/PDMS, was reported to be very high for extracting VOCs. However, competitive adsorption and displacement effects make mass calibration and quantification particularly challenging. The solid coatings can extract (via adsorption) great amounts of VOCs, but short sampling times and nonequilibrium conditions have to be used and

operating conditions must be carefully adjusted too [17]. Presented inside needle capillary adsorption trap (INCAT) could overcome these disadvantages.

When sweet wines are analysed by direct injection, due to their high content in sugar and to the high temperature in the injector and in the column, the caramelization of sugars is possible, causing irreversible damage of the column, especially capillary columns. Additionally, the injection of wine samples produces a great amount of particles that can plug column tips causing variation in carrier fluxes and peak shapes [1]. In these cases the INCAT technique [18] can represent a better alternative for column protection. In relation to other extraction techniques used in wine analysis the INCAT technique offers many advantages because it does not require solvent or any sample treatment. Moreover it is fast, inexpensive and requires low sample volumes. The main advantages of this method lies particularly in the possibility of sampling directly in the field, and very short sampling time up to two minutes. The INCAT sampling method was verified, optimized, validated and compared to purge-and-trap technique [18] as well as to SPME [17] in our previous paper [17, 18].

In this work, an INCAT method to determine major compounds such as higher alcohols, esters and other major compounds in white wine has been applied to Slovak wine (Italico Riesling, Modra region).

II. EXPERIMENTAL

2.1 Materials

Ethanol (96 %) was from LCHM-Labochem (Bratislava, Slovakia). Carboxen 1000 60/80 mesh and aluminum oxide with grain size of 0.2-0.4 mm as packed materials for needle concentrator were purchased from Supelco (Bellefonte, PA, USA) and Merck (Darmstadt, Germany), respectively. The chemical standards isobutanol, 3-methyl-1-butanol, propyl acetate, butyl acetate, amyl acetate, citronellol, geraniol, linalool, α-terpineol were purchased from Ultra Scientific (N. Kingstown, RI, USA); isoamyl acetate, ethyl octanoate, ethyl decanoate, ethylhexanoate were purchased from Merck (Hohenbrunn, Germany); n-amyl alcohol, isobutyl acetate, 2-phenylethyl alcohol were purchased from Chem Service (West Chester, PA, USA); 1-propanol, isopropanol, hexanol, heptanol, octanol, benzyl alcohol, ethyl acetate were purchased from Lachema (Brno, Czech Republic); nerol was from Aldrich (St. Louis, MO, USA). A water standard solution containing 12 % ethanol (v/v) was prepared by diluting of ethanol standard solution containing 100 µl/L of listed chemical standards. Wine samples (vintage 2002 and 2003) were from Modra region (Jaroslav Bibza, Modra, Slovakia).

Stainless steel needles (cannula) 90 mm long with an outher diameter (o.d.)/inner diameter (i.d.) of 1.3/1.1 mm, and 1.1/0.9 mm were from Nissho (Osaka, Japan), and from this material were prepared also O-rings (3 mm x 1.1 mm o.d./0.9 mm i.d.). Stainless steel frits (20 µm porosity and 0.16 mm depth) were from Carlo Erba (Milano, Italy), glass microliter syringe for sampling (200 µl) from Hamilton (Bonaduz, Switzerland), and viton tubing 20 mm x 5 mm o.d./0.9 i.d. from Masterflex (Vernon Hills, IL, USA).

Capillary column DB-1 30 m x 0.53 mm i.d. x 2,65 µm from J&W Scientific (Blue Ravine Road, Folsom, USA) was employed for analytical separation of desorbed wine components.

2.2 Wine making

Sound white grapes of Italico Riesling (2000 kg) were obtained from the vineyard in Modra region during the 2002 and 2003 vintages and transported to the experimental winery at the Bibza Modra, Slovak Republic in 20 kg plastic boxes. The grapes were destemmed and crusched on a commercial grape destemmer-cruscher and then transferred into a wooden tank for maceration and treated with sulfur dioxide (20 mg/kg). The maceration time was 12 hours at about 25 °C. During this time the mixture was stirred twice to increase the extraction of polyphenolic and aroma compounds. After maceration time was completed, pomaces were pressed gently in a horizontal press. The musts were fermented (25 °C) with spontaneous yeasts in two polypropylene tanks (600 l). After the alcoholic fermentation, the young wines were allowed to stand for malolactic fermentation (20 °C). The wines were then racked and added with sulfur dioxide (75 mg/l). They were stored at 15 °C in polypropylene tank prior to analysis.

2.3 GC analysis

The GC measurements were performed using gas chromatograph HP 6890 Hewlett-Packard (Avondale, USA) equipped with a split-splitless injector, and mass spectrometric (MS) detector 5973 Network Agilent Technologies. The carrier gas was helium with pressure of 20 kPa in the injection port. The injection was made in split mode with a 10:1 split ratio, using a liner of 5 mm i.d., at injection port port temperature 320 °C, and an oven temperature programme of 25 °C, 3 °C/min, 200 °C. A 31-350 m/z mass range was recorded in SCAN mode for identification, and selected iones 31, 41, 42, 43, 45, 46, 55, 56, 60, 69, 70, 73, 75, 79, 86, 88, 91, 93, 96, 108, 122 m/z mass were recorded in SIM mode for quantification.

III. RESULTS AND DISCUSSION

An INCAT device with adsorbent inside of the whole volume of stainless steel needle was developed for the pre-concentration of trace volatile organic compounds from aqueous samples. As can be seen from Fig. 1 it comprises a stainless steel needle N, stainless steel O-ring O, stainless steel frits F, in laboratory prepared shut-off micro valve V with stainless steel body and viton tubing T, adsorbent Carboxen 1000 60/80 mesh (50 mm length of needle) C, and alumina 0.2 – 0.4 mm (7 mm length of needle) A.

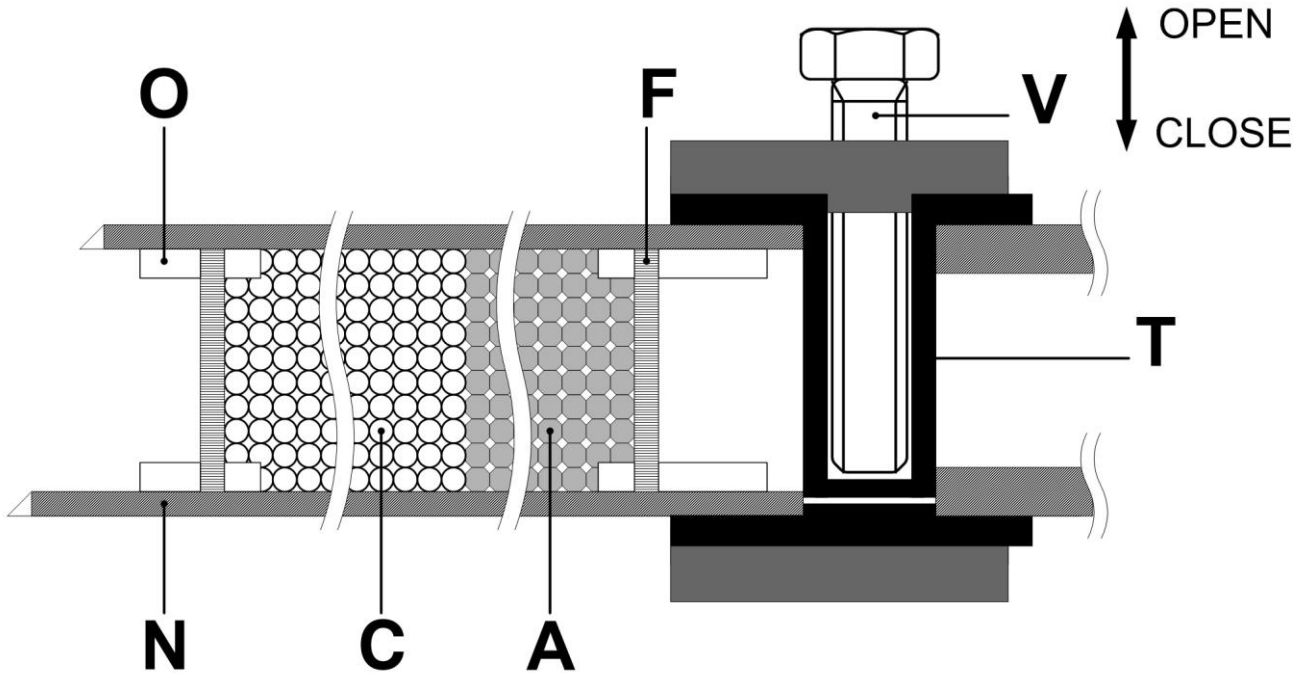

FIGURE 1 SCHEME OF INCAT SYSTEM [17, 18]. (N) STAINLESS STEEL NEEDLE; (O) STAINLESS STEEL O-RING; (F) STAINLESS STEEL FRITS; (C) ADSORBENT CARBOXEN 1000; (A) ADSORBENT ALUMINA; (V) SHUT-OFF MICRO VALVE WITH STAINLESS STEEL BODY; AND (T) VITON TUBING.

Fig. 2 represents single steps of adsorption and desorption of analytes from INCAT device. Wine sample is passed through the INCAT device by means of microliter syringe with 100 µl volume at speed about 50 µl/min and analytes are retained in INCAT device (Fig. 2A). Subsequently the INCAT device is flushed by cca 0.1 ml of distillated water to wet alumina (Fig. 2B) and then by cca 0.5 ml of air to remove residual water (approx. of 20 µl) (Fig. 2C) at room temperature. Then the valve is closed and the INCAT device is introduced to GC injection port with 1 ml liner in the split mode at 320 °C. Adsorbed analytes are thermally desorbed from Carboxen 1000 and displaced to the injection port with gradually purging water steam formed by evaporation of water (approx. of 9 µl determined by weighing of alumina sorbent). Various materials were tested for the water reservoir in needle concentrator, e.g. silica and molecular sieve, but the alumina was found to be most suitable. Desorbed analytes are separated by capillary gas chromatography and detected by FID or MS detector (Fig. 2D). Once

analysis is finished the valve on INCAT device is open and residual organic compounds are removed by stream of helium with flow of 70 ml/min within 2 minutes (Fig. 2E).

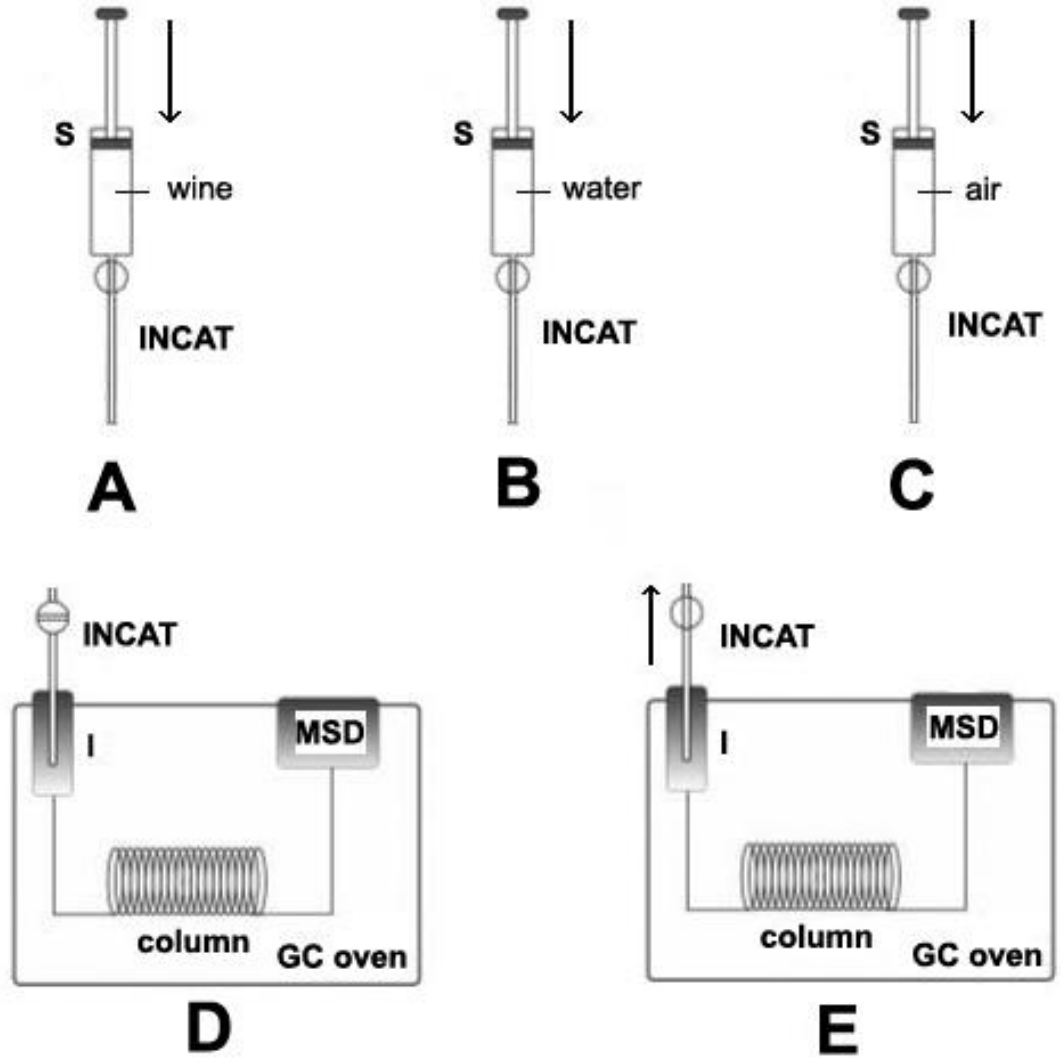

FIGURE 2 SCHEME OF SORPTION AND DESORPTION PROCESS OF INCAT DEVICE. A – SAMPLING OF WINE SAMPLES, B – FLUSHING OF INCAT DEVICE BY WATER TO REMOVE RESIDUAL WINE AND WET ALUMINA, C – REMOVING OF RESIDUAL WATER, D – DESORPTION, E – CLEANING. S - SYRINGE; MSD – MASS SPECTROMETRIC DETECTOR; I - INJECTOR.

In contrast with the other INCAT systems published previously [19], the proposed needle concentrator is characterized by closed sorption-desorption system in which thermally desorbed analytes are with the assistance of water vapors repelled to the injection port of gas chromatograph without additive make-up gas. This modification allows using this needle concentrator for each type of gas chromatograph with split-splitless injector without any modification.

Fig. 3 shows chromatogram of a wine at sampling volume 100 µl using INCAT technique.

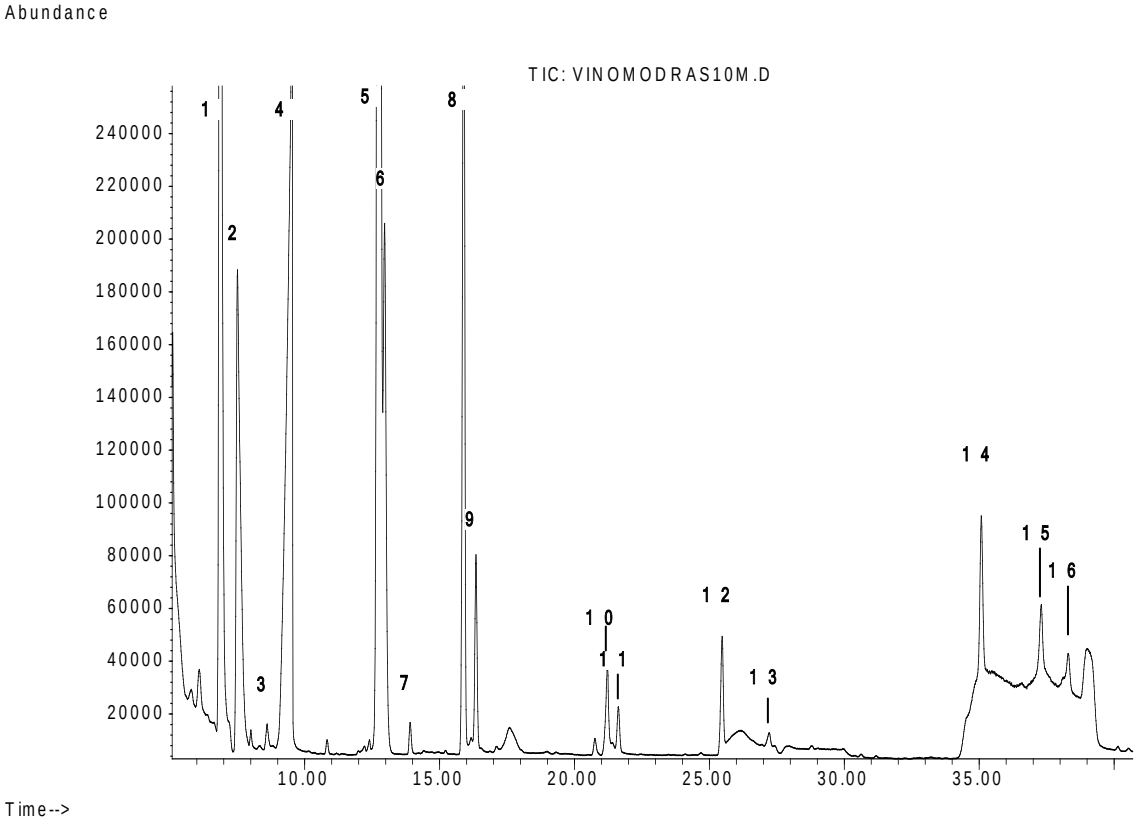

FIGURE 3. GC-MS/SIM CHROMATOGRAM OF A WHITE WINE AT SAMPLING VOLUME 100 ML USING INCAT DEVICE. (1) ethylacetate; (2) i-butanol; (3) formic acid; (4) acetic acid; (5) i-amylalcohol; (6) 1-butanol–2-methyl; (7) propyleneglycol; (8) 2,3-butanediol; (9) 1,3-butanediol; (10) i-amylacetate; (11) butyrolactone; (12) succinic anhydrid; (13) hexanoic acid; (14) 2-phenylethylalcohol; (15) ethylhydrogensuccinate; (16) octanoic acid.

IV. CONCLUSION

A simple method of solventless extraction of volatile organic compounds from liquid samples has been applied to wine samples. In relation to other extraction techniques used in wine analysis the INCAT offers many advantages because it does not require solvent or any sample treatment. The main advantages of this method lies particularly in the possibility of sampling directly in the field, and very short sampling time up to two minutes.

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