ANALYSIS OF ALCOHOLS IN BLOOD AND BLOOD SERUM

Abstract

A Head Space analysis method is validated for the determination of ethanol in blood samples. A Konik K-MAS5 Autosampler Head Space is used for a routine analysis.

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Introduction

Gas chromatographic procedures have already been established as officially recognized methods for the determination of alcohol in blood and other biological samples. Gas chromatography is specific, rapid, precise and requires no reagents, and also has the advantage that other volatile substances present in the sample can simultaneously be determinate.

Routine blood alcohol analysis by Head Space-Gas chromatography is the method of choice since current government regulations are reducing the ethanol limits in blood. In Spain, for instance, government regulations dictate that ethanol in blood has to be lower than 0.5g/L in blood, or 2.5mg/L in breathed air (Real Decreto 13/1992 - Chapter IV).

The analysis of the vapor phase over the solution at a predetermined Equilibrium Temperature (Head space) eliminates the need for alternatively complex sample preparation and only the internal standard has to be added. With the Head Space technique, only the vapor phase in equilibrium with the sample is injected, eliminating interferences of other substances presents in the sample.

Requirements for this analysis include the identification and quantification of all analytes with a short turnaround time. This can be performed with the use of autosampler systems in order to optimize the precision and reproducibility of the analysis at the same time as the total time is clearly decreased, as next sample conditioning can take place while the previous sample is being analyzed.

Validation of the analysis of volatile compounds in blood samples and in serum samples are performed. The volatile compounds determinated are: Methanol, Ethanol, Acetone, Isopropanol and n-Propanol. This Konik Application Report presents the results of the validated methods for the determination of ethanol in both matrixes: human blood and blood serum.

Table 1: **Chromatographic conditions**

GC	Column:	J&W- DB-624, 30m, 0.53mm, 3µm (ref.: 125-1334)
	Carrier:	He; constant Pressure 10psi
	Injector:	250ºC, inj. mode: conventional injector
	Oven:	30ºC Isothermal
	Detector:	250ºC; detector gases: H_2 at 39ml/min; air at 220ml/min; N_2 (Make-up) at 25ml/min
Headspace:	Tray:	32vials of 10ml; 10ºC
	T desorb:	80ºC
	T line:	160ºC
	T valve:	160ºC
	t desorb:	10min
	t inj:	20s
	Cleaning gas:	He 8psi

Experimental

Konik HRGC 4000B Gas Chromatography System was used in this analysis. Control of GC is done using Konikontrol®. Data acquisition, reduction, and analysis were done using Konikrom® Chromatography Data system.

Head Space sampling was performed with the Konik K-MAS5 Multimode Autosampler in the headspace mode. 32 sample tray was used for the maximum automatization. Its "intelligent programmation" allows the minimum analysis time as the limiting factor is the time of the chromatogram run.

The optional TTC (Tray Temperature Control) module is used in order to keep the biological sample at the properly temperature (10ºC) to avoid sample degradation while the automated analysis was done.

The transfer line was connected directly into the GC injector through a proper connection. Conventional injector mode was used in the Konik Multimode split-splitless injector.

Standard Preparation
Standard solution (1000µg/ml of Methanol, Ethanol, Acetone, Isopropanol and n-Propanol) was prepared mixing 50mg of each standard with 50ml of wate (HPLC grade).

Sample Preparation
A minimum of 0.5 ml of serum, plasma, whole blood or urine is required. Sample should be stored at 4ºC if not analyzed within 2-4 hours.

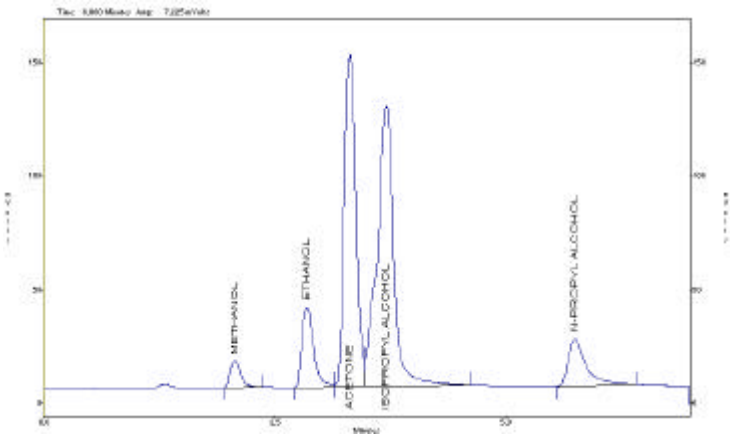
For trace analysis of volatile substance, salts can be added to the aqueous solutions in order to increase the vapor pressure of analytes of interest. This is more necessary when the same are very soluble in water matrixes.

Biological samples are prepared into 10ml magnetic crimped vials. Previously, sodium sulphate is added to each autosampler vial (aprox. 2g). 0.5ml of sample (blood or serum) is added to each vial. Subsequently vials are spiked with 40 to 60ul of standard solution to obtain 80 to 120 µg/ml volatiles spiked water and crimped immediately.

Instrument conditions: The instrument conditions are listed in Table 1 (page 1).

Results
Figure 1 shows the Head Space chromatogram obtained in this analysis.

Figure 1:
HeadSpace chromatogram of 0.5ml of blood spiked with 100 µg/ml of standard solution.



Both methods: determination of alcohol in blood and determination of alcohol in serum were performed. Specificity, precision, linearity and accuracy parameters have been calculated with a good results.

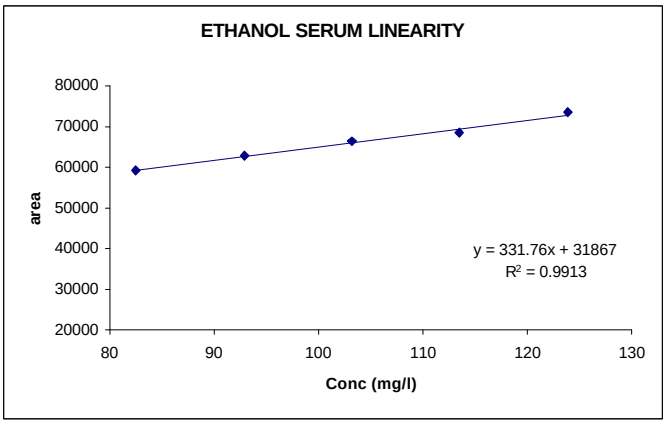
Table 2 shows the precision results obtained for the determination of ethanol in blood and in serum matrixes. The retention time reproducibility is very good. The absolute areas measurements obtained will be significantly improved using an internal standard.

Figure 2 shows the linearity graphic obtained for the analysis of ethanol in serum.

Table 2: **Precision for area and retention time (n=6)**

	BLOOD		SERUM	
RUN	**Retention Time (min)**	**Area**	**Retention Time (min)**	**Area**
1	2.880	117140	2.897	711174
2	2.900	118067	2.897	665923
3	2.900	122201	2.891	628913
4	2.890	115970	2.897	767902
5	2.900	105703	2.909	684891
6	2.880	103546	2.903	735127
Mean	2.892	113771	2.899	698988
Std	0.0098	7420.9	0.00620	49812.0
% RSD	**0.34**	**6.5**	**0.21**	**7.13**

Figure 1: **Linearity graphic of ethanol spiked serum (80 to 120 µg/ml)**



Conclusions

Validation is performed for each compound (both in blood and in serum matrixes) with as good results. So Konik K-MAS5 Multimode Autosampler System in Head Space mode is an excellent system for the routine determination of alcohol in biological samples.

This procedure is mainly recommended for the analysis of a wide range of volatile compounds in biological samples.

References

- Real Decreto 13/1992 - 17 de Enero.
- "*Sensitive Head-Space Gas Chromatographic Method for the Determination of Ethanol Utilizing Capillary Blood Samples*". Analytical Chemistry, Vol 47, Nº 9, Augoust 1975.
- "*Determination of Alcohol in Blood by Gas Chromatographic Head space Analysis*". Clinical Chemistry Newsletter, Vol 4, Nº 2, 1972.