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Short Communication

Determination of ethanol in biological samples by head-space gas chromatography*

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Introduction

Alcohol exposure during gestation results in a wide range of abnormalities in children; these abnormalities have been collectively labelled the faetal alcohol syndrome (FAS) [1]. At present, FAS is one of the most frequently recognizable causes of mental and growth retardation in humans.

The chick embryo is a widely-used model for testing teratogenic and neurotoxic effects because of its sensitivity to many exogenous agents and because the anatomy, embryology and physiology of the avian system have been extensively studied. Exposure of early chick embryos to ethanol has been known to cause complex and often subtle embryopathic effects similar to FAS [2].

A number of methods have been reported for the determination of ethanol in blood samples [3–6], however few chromatographic methods are available for the analysis of ethanol in other biological samples [7]. A sensitive and accurate head-space gas chromatographic method for the measurement of ethanol in different tissues of the chick embryo is described.

Materials and Methods

Biological samples

Fertile eggs (Shaver Star-cross 288) were incubated at 37.7°C in 70–80% humidity in a forced-air incubator, with turning every hour. Ethanol (50%, 1.362 g kg^{-1} egg) was injected into the air space of the eggs at the start of the incubation (day 0) using distilled water as the vehicle. Vehicle-treated embryos received distilled water only, and sedentary eggs were incubated to control for seasonal variations in fertility and viability for all groups. A total volume of 200 μl was used for both vehicle and vehicle + ethanol injections. Embryos were killed on days 4, 6, 8, 11, 14 and 20 of incubation and the biological samples were analysed without delay. The first sampling was on day 4; each sample consisted of a pool of 7–8 embryos.

Reagents

The chemicals used were of analytical reagent grade and the water was double distilled. Ethanol reference standards of 0.79 mg ml^{-1} were prepared from absolute alcohol (E. Merck). The aqueous internal standard solution contained 0.5 mg ml^{-1} *n*-propanol (E. Merck).

Instrument

A Perkin–Elmer model Sigma 300 gas chromatograph (GC) equipped with a flame ionization detector and a head-space sampler HS-6 was used. The operating conditions were as follows: the stainless steel column measuring 2 m × 1/8 in. i.d. was packed with 15% carbowax 1500 on chromosorb W acid washed (80–

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100 mesh). The column temperature was 100°C with injector and detector temperatures of 150 and 200°C, respectively. Carrier gas (nitrogen) was used at a flow rate of 30 ml min^{-1}. A head-space sampling technique [6] was used for the analysis.

Sample treatment

Tissue samples were treated without delay as follows: homogenization with distilled water (1:10, w/v) and centrifugation (4000*g*) at 4°C. The supernatant was used for the ethanol analysis. Amniotic fluid samples were analysed without pre-treatment.

Analytical procedure

Aliquots of 1.0 ml of previously treated samples were transferred into head-space vials and 100 μl of the internal standard solution was then added. After incubation at 60°C for 30 min, the vials were automatically sampled (injection time, 5 s) and chromatographed under the above mentioned conditions.

Results and Discussion

Sample homogenization

In the preliminary trials, tissue samples from chick embryos previously injected with ethanol were homogenized with 0.6 N perchloric acid (PCA) (1:10, w/v) [7] or distilled water (1:10, w/v). Because there was no significant difference between the results obtained with and without protein precipitation (Table 1), homogenization with distilled water was selected.

Table 1
Effect of sample homogenization solvent on ethanol determination

Sample	Ethanol conc. ±SD (mg g^{-1})*	
	PCA	Water
Embryo pool†	0.95 ± 0.03	0.96 ± 0.02
Brain‡	0.76 ± 0.01	0.76 ± 0.01
Liver‡	0.63 ± 0.02	0.77 ± 0.01

*The data represent the average of four measurements.
†4 days of development; ethanol injection: day 0.
‡14 days of development; ethanol injection: day 13.

Linearity and limit of determination

Linearity was observed over the range 5–150 μg ml^{-1} ethanol. The equation $A/A_{is} = 0.017 + 0.0115c$ ($r = 0.9997$) held, where A/A_{is} is the ratio of peak areas for ethanol–*n*-propanol, and c is the concentration of ethanol in μg ml^{-1}.

The determination limit, defined as the lowest concentration resulting in a signal-to-noise ratio of 4, was 5 μg ml^{-1} ethanol.

Table 2
Method reproducibility

Conc. (μg ml^{-1})	Relative standard deviation (%)	
	Within-day (n = 10)	Between-day (n = 5)
40	3.2	6.1
100	2.7	3.4

Reproducibility

Both between- and within-day reproducibilities were evaluated from the analysis of replicate samples for different concentrations. The resulting relative standard deviations (summarized in Table 2) showed the method to be precise.

Ethanol recovery from biological samples

Ethanol recovery was determined from control chick embryo samples of different days of development (complete embryo or head pool of 6 day embryos; brain or liver samples from

Table 3
Recovery of ethanol in biological samples*

Sample	Days of development	Ethanol added (mg g^{-1})	Recovery ± SD (%)	RSD (%)
Embryo	6	0.4	88 ± 4	4.5
		1.0	88 ± 2	2.0
Head pool	6	1.0	92 ± 3	2.8
Brain	20	0.4	90 ± 6	6.6
		1.0	80 ± 2	2.5
Liver	20	0.4	89 ± 5	5.6
		1.0	81 ± 4	4.9

*The data represent the average of three samples (in triplicate). Ethanol was added to control embryo samples before homogenization.

20 day embryos). Biological samples were spiked with ethanol before homogenization. The results were between 84–96 and 78–95% for 0.4 and 1.0 mg g^{-1} ethanol added (Table 3).

Determination of ethanol in chick embryo

The method validated here was applied to the monitoring of ethanol in chick embryos treated with ethanol as described in the Materials and Methods section. After ethanol injection, the maximum ethanol concentration in chick embryos for all subjects was 0.96 mg g^{-1}, observed on day 4 of incubation (the first sampling). It then decreased slowly to below the determination limit on days 11–14 of development. However, the ethanol concentration in amniotic fluid on these days ranged from 40 μg ml^{-1} and undetectable (Table 4).

Table 4
Ethanol concentration in chick embryo samples*

Sample	Days of development	Ethanol conc. ± SD (mg g^{-1})
Embryo pool	4	0.96 ± 0.02
Embryo pool	6	0.88 ± 0.04
Head	8	0.58 ± 0.03
Body	8	0.58 ± 0.02
Brain pool	11	0.05 ± 0.03
Amniotic fluid†	11	(40.1–9.0)
Brain	14	ND
Amniotic fluid†	14	(6.0–ND)
Brain	20	ND
Amniotic fluid†	20	ND

*The data represent the average of 4–6 samples (in triplicate).
†Without pre-treatment (mg l^{-1}).
ND = not detected or below the determination limit.

Conclusion

Ethanol can be determined efficiently in biological samples using the head-space gas chromatographic technique. The proposed method was applied successfully to the monitoring of ethanol in treated chick embryos, using water as the medium for homogenization of samples. The analytical characteristics of the method are satisfactory for routine biomedical applications.

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