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Short communication

Contamination in HPLC quantified benzaldehyde is from polypropylene microtubes

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Abstract

Semicarbazide-sensitive amine oxidase (SSAO; EC 1.4.3.6) is a copper-containing enzyme predominantly expressed by vascular smooth muscle cells. SSAO deaminates primary amines to produce aldehydes and oxygen peroxides, and may thus play a role in vascular damage. SSAO activity can be quantified by assaying benzaldehyde production using fluorescent derivatisation and separation by HPLC. We performed the derivatisation step in polypropylene or borosilicate glass tubes over 45 min at 95 °C. High and obstructing background levels of benzaldehyde were found in one batch of polypropylene vials, as opposed to its alternatives. Treatment and handling of product shipment into the country did not account for introduction of contaminant into packaged vials nor did any reagent used in the assay. We conclude that the source of contamination was most likely due to variation in the commercial production process. Use of borosilicate vials for assays based on aldehyde production and derivatisation is recommended.

Keywords: Semicarbazide-sensitive amine oxidase; HPLC; Benzaldehyde

Semicarbazide-sensitive amine oxidase (SSAO; EC 1.4.3.6) is a copper-containing enzyme secreted in vivo predominantly by vascular smooth muscle cells [1]. SSAO deaminates amines to produce aldehydes and oxygen peroxides, and may thus play a role in vascular damage. Elevated SSAO levels have been reported in type-1 and type-2 diabetes patients in association with coronary artery disease [2]. Of the available methods for the determination of SSAO activity in human plasma, a relatively simple and sensitive HPLC method with fluorimetric detection of aldehyde products has been reported [3]. Quantification of SSAO activity is achieved by adding a known amine substrate (benzylamine) and detecting its aldehyde product (benzaldehyde) following deamination by SSAO in a plasma sample, previously treated with a monoamine oxidase inhibitor and incubated for 60 min at 37 °C. Samples were subsequently derivatised by incubation with dimedone and sulphuric acid at 95 °C for 45 min. This incubation was carried out in polypropylene microtubes imported from USA.

Using the method by van Dijk et al. (1995) we produced similar chromatograms using the following Waters® HPLC system: 717_{plus} Autosampler, 474 Scanning Fluorescence Detector, 510 Pump with an In-Line Degasser AF, Waters® Atlantis column (dC_{18} 3 μm, 4.6 mm × 150 mm) and Empower Pro software (ver. 5.0, Waters Corp. 2002). Twenty microliters of each sample was injected onto the system with a mobile phase consisting of acetonitrile–water (55:45, v/v) at a flow rate of 1.0 ml/min. The spectrofluorimeter was set at $\lambda_{Excitation}$ = 386 nm and $\lambda_{Emission}$ = 451 nm and the room temperature was 25 °C. An additional peak located towards the end of each sample measurement time was detected in all polypropylene microtubes (Fig. 1A and C; peaks #2), which did not interfere with the assay. Additionally, there was a minor background peak with the same retention time as benzaldehyde but this was not large enough to affect the analytical properties of the assay (data not shown). However, in another batch of polypropylene microtubes supplied later in a calendar year,

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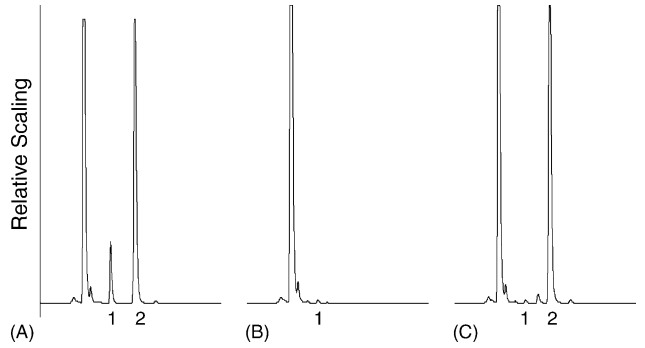

Fig. 1. Blank samples extracted in contaminated polypropylene microtube (A), borosilicate vial (B) and polypropylene microtube free of contaminant (C). *Legend*: (1) Represents benzaldehyde and (2) represents additional peak identified in all samples extracted in polypropylene microtubes.

very high and obstructive background levels of measured benzaldehyde were apparent (Fig. 1A, peak #1). Using borosilicate vials (Waters®; 1.0 ml clear glass shell vials with polyethylene snap cap) for the derivatisation step, we attempted to isolate the source of this contamination by derivatising each reagent used in the assay but found no interfering peaks in any reagent or when the full assay protocol was implemented (Fig. 1B). Only when polypropylene microtubes were used did the interfering peak appear (Fig. 1A, peak #1). In a subsequent use of a different batch of polypropylene microtubes, supplied on request from the manufacturer and not subjected to chemical treatment during importation (see below), no interfering peak was observed (Fig. 1C, peak #1). This led to the assumption that the source of contaminant is either from handling of the equipment between departure from the US and arrival in Australia or that the production methods for polypropylene microtubes were modified. Goods arriving in Australia by air or sea are usually treated against potential pests and as such considered to be the only outstanding procedure that has the potential to introduce a contaminant. However, the Australian Quarantine and Inspection Service (website: http://www.affa.gov.au/) uses methyl bromide (48 g/m^3/24 h, temperature 21–24 °C) and sulphuryl fluoride (64 g/m^3/16 h, temperature 21–24 °C) as conventional pesticides, both are highly reactive non-aldehyde agents that are less likely to react with the packaged microtubes to cause an adverse effect. Another potential source of apparent obstructive background levels lies within minor modifications of the product itself that is responsible for the undesired effect.

We therefore strongly recommend the use of glass vials instead of polypropylene microtubes in SSAO activity assays involving derivatisation of benzaldehyde. If the use of glass tubes is undesirable for other reasons, we suggest requesting a full history of the polypropylene microtubes of each batch supplied by companies importing laboratory equipment into Australia, including batch handling and quality control information.

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