spective gel image revealed that the density of the area between 200 and 1600 bp was very similar in all of the four lanes. Therefore, volume-corrected calculation of this experiment (Fig. 1B, filled columns) displayed results comparable to the densitometric analysis of Fig. 1A (shown in Fig. 1B, open columns). Thus, this method offers reproducible results as a function of cell number but independent of the volume loaded on the gel.

To study the detection limits of this new method we further decreased the cell number (Fig. 2). GH_3 cells (0.25×10^6, 0.125×10^6, and 62,500) were treated with okadaic acid and apoptotic DNA fragments were isolated as mentioned above. The TE volume to elute the DNA from the miniprep column was decreased to 30 μl and the eluate was completely loaded on the agarose gel. Distinct DNA bands were clearly visible at cell numbers of 0.25×10^6 (Fig. 2A, lane 6) and 0.125×10^6 (lane 4), while the laddering from 62,500 treated cells appeared very faint (lane 2). In contrast to the optical impression, densitometric scanning of the gel image revealed an OD intensity of specific as well as background signals in good agreement to the cell number (Fig. 2B) even at 62,500 cells. Thus, additional densitometric analysis showed successful discrimination of the bands between 200 and 1600 bp at all three cell numbers (Fig. 2C).

In conclusion, the use of modified lysis and precipitation conditions allowed the successful rapid isolation of visible discrete apoptotic DNA fragments from as less as 0.125×10^6 cells. Densitometric analysis of the laddering was even more sensitive and allowed analysis down to 62,500 cells. With this modified miniprep spin column protocol at least 12 samples can be processed in about 1 h. Further experiments have shown that the technique is successfully applicable to the study of drug-induced apoptosis in other adherent cell lines as well as cells growing in suspension cultures.

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A Fast and Inexpensive Procedure for Drying Polyacrylamide Gels

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Reproducible drying of polyacrylamide slab gels usually requires the availability of expensive commercial gel driers which typically include a vacuum system and a heater. Alternatively, the gel is equilibrated in glycerol and drying takes place overnight between two sheets of cellophane membrane (1). In this case, extra care must be taken to avoid trapping air bubbles both at the gel/cellophane interface and around the gel edges, or gel cracking will result.

Here we describe a simple alternative procedure which only involves soaking the gel twice in low-grade ethanol: The gel is placed in a petri dish containing 5–10 gel volumes of any suitable type of low-grade (95% v/v) ethanol and stirred for 10-15 min. After that time, the ethanol solution is refreshed and soaking continues for 5 min. During this second soak the gel becomes opaque, dehydrates, and shrinks uniformly (without cracking) by a factor of about 35–40%. In the final step, the gel is removed from the ethanol solution and placed on a hard (nonadhesive) surface, ethanol is allowed to evaporate from its top surface, and then it is covered with a glass plate to avoid curling during the final stages of ethanol evaporation. After a few hours the gel is ready for storage.

Figure 1 compares the same gel before and after drying. Although gel shrinkage is usually considered a disadvantage, it does increase the effective sensitivity of the method, since bands that would be too weak to detect on the full-size gel now become detectable. The sensitivity is also increased by the loss of gel transparency. Gels dried with this procedure become sensitive to moisture, but are otherwise stable. The dried gels can be stored by any suitable method, such as between cellophane sheets or (in the case of minigels) inside slide holders. The method has been tested with minigels with size up to 12×12 cm, and with polyacrylamide concentrations ranging from 7.5 to 15%.

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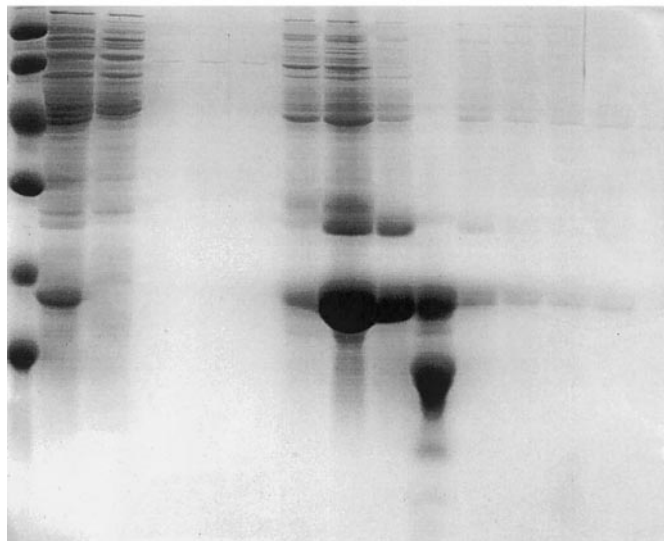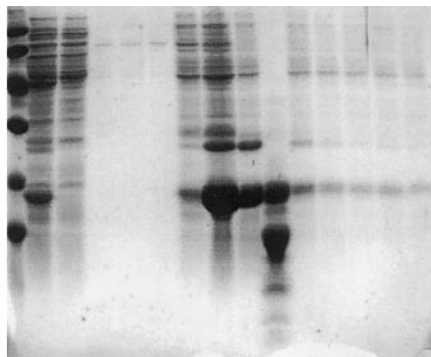
FIG. 1. Comparison of a polyacrylamide slab gel before (bottom) and after (top) the application of the drying procedure. The size of the original gel is $92 \times 76 \times 0.7$ mm^3. Note the increase in the intensity of the weak bands in the dried gel.

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Enantiomeric Separation of Hydroxy
Eicosanoids by Chiral Column
Chromatography: Effect of
the Alcohol Modifier[1]

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Steric analysis of hydroxyeicosatetraenoates (HETEs)[3] and other fatty acid derivatives plays a vital part in the characterization of lipoxygenase, cyclooxygenase, and P450 reactions, and there is also often a need for preparative resolution of oxylipin and eicosanoid enantiomers. Assignment of the absolute configuration can be accomplished using spectroscopic techniques such as NMR (1) and circular dichroism (2). However, chiral column chromatography represents one of the most straightforward methods, and it can be performed with basic HPLC equipment. The first generation of chiral columns gave marginal resolution of HETE enantiomers (3), and for many compounds derivatization was required to achieve baseline resolution (e.g., (4)). More recent chiral columns, particularly of the Chiralcel class of derivatized cellulose-based supports, can achieve much improved resolution of individual hydroxy eicosanoids (5). A problem is that an individual column type is capable of separating a limited range of racemic eicosanoids. For example, the Chiralcel OD column, perhaps the most versatile of the Chiralcel class, will resolve 8-HETE and 12-HETE, whereas 15-HETE is not well resolved. Also, preparative separations require more than the minimal baseline resolution that is adequate for analytical work. Overall, a battery of chiral columns is required to allow the investigator to deal with a wide variety of fatty acid derivatives.

After we had been unable to resolve a tosylated hydroxystearate using chiral-phase HPLC (including testing methyl and pentafluorobenzyl ester derivatives (6, 7)), the technical service at Chiral Technologies (Exton, PA) screened the sample on their selection of columns and reported baseline resolution of the methyl ester using a Chiralpak AD column and a solvent of hexane/ethanol (100/2, by vol). Subsequently, we found that this separation was completely dependent on the use of ethanol as opposed to isopropanol as the alcohol modifier. Effects of the alcohol modifier on chiral separations have been reported for several classes of aromatics and pharmaceuticals (e.g., (8, 9)). Here we report that with the Chiralpak AD column there are striking improvements in separation with a wide variety of lipoxygenase products and other hydroxy derivatives on changing the alcohol modifier from isopropanol to ethanol or to methanol.

Racemic HETEs and HODEs were synthesized by autooxidation of arachidonic acid or linoleic acid, respectively, in the presence of α-tocopherol (10). Follow-

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[3] Abbreviations used: AUFS, absorbance units full scale; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; PG, prostaglandin.