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# A COMPLETE SEPARATION OF MAJOR PHOSPHOLIPID CLASSES IN MEAT BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH SUBSEQUENT GASCHROMATOGRAPHICAL ANALYSIS OF PHOSPHOLIPID-BOUND FATTY ACIDS

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## SUMMARY

Phospholipids (PL) are of increasing interest in basic investigations of physiological, anatomical and technological properties of muscle and meat.
The major disadvantages of the usual separation by thin-layer-chromatography are the limited capacity and the very high amount of labour when the different PL fractions must be collected for further analysis.
From several high performance liquid chromatography (HPLC) methods already used for single PL or incomplete separation, a fast and efficient HPLC separation of all major classes was developped. Baseline separation was achieved for Phosphatidyl-Choline (PC), Phosphatidyl-Ethanolamine (PE), Phosphatidyl-Serine (PS), Phosphatidyl-Inositol (PI), Cardiolipin (C), Sphingomyelin (S), Lyso-Phosphatidyl-Choline (LPC) and Lyso-Phosphatidyl-Ethanolamine (LPE) in less than 50 minutes. It was possible to collect automatically the single phospholipid fractions. Retention time and peak areas were highly reproducible also from the Fatty Acid Methyl Esters (FAMES) pattern of the phospholipid classes analysed by Gaschromatography (GC).
This method requires no derivatisation, cleaning steps or concentration procedures of the sample compounds. Further transesterification of the phospholipid bound fatty acids and GC analysis with internal standard allows the recalculation of the total amount of each PL and of the whole PL pattern in the sample.

## INTRODUCTION

In raw or processed meat as well as in blood samples and biopsies the phospholipid pattern becomes increasingly interesting. While phospholipids are always associated with other lipids, the separation and quantitation out of complex samples is difficult, especially when some phospholipid fractions occur in disproportional small amounts (CHRISTIE, 1985).
When further analysis of individual PL classes is intended, the usual thin-layer-chromatographic methods show early limits in their resolution capacity, they are cumbersome and time consuming. Based on some HPLC procedures (HURST et al., 1984; NISSEN et al., 1983) a sensitive HPLC-method for the separation of all major PL-classes was developped, which allows the automatic collection of the fractions for further transesterification and gaschromatographic determination of the indi- vidual PL-fatty acid pattern.

## MATERIAL AND METHODS

Total lipids from pig longissimus dorsi muscle were extracted 1 h post mortem by chloroform/methanol (HALLERMAYER, 1976). 4 mg of the lipid extract were injected in the 20 µl sample loop of the HPLC-unit (MERCK-HITACHI, Darmstadt, FRG). Stationary phase was silica (Si 60, 5µm, 250x4 mm LiChrocard; MERCK, Darmstadt, FRG), and the mobile phase consisted of acetonitril, methanol and phosphoric acid. The solvent system is based on the simultaneous use of a pH-gradient in a polarity gradient: after 5 min. acetonitril the mobile phase changed to acetonitril containing 3 ‰ phosphoric acid; from the 15th min. to the 30th min. this system changed continually to methanol also containing 3 ‰ phosphoric acid. The flow was held constant at 1 ml/min. All PL-standards were obtained by SIGMA (Taufkirchen, FRG) and all chemicals by MERCK (Darmstadt, FRG). Phospholipids were detected at 203 nm, the individual fractions were collected by a GILSON Fraction Collector (Villiers-le-Bel, France). Within each fraction the PL bound fatty acids were transesterified (SHEHATA et al., 1970) to FAMES and analysed by GC (HEWLETT PACKARD GC 5791, Waldbronn, FRG) on a capillary column (ICT, Durabond Wax, 30 m x 0,25 mm), the carrier gas was $H_2$ with a flow of 2 ml/min and a split of 1:40. The temperature program started at 94°C with an increase of 10°C/min, 250 °C were held for 5 min.

## RESULTS AND DISCUSSION

Separation of all major PL classes was performed at the baseline level in less than 50 minutes (Fig.1). As the fatty acid pattern of PL classes are changing and the sensitivity of an UV detector depends mostly on the degree of unsaturation in the PL, a quantitation by UV detector might not be correct. However the use of PL-standards with the same fatty acid pattern gave linear and reproducible calibration graphs for all PL within a range from 1 µg to 40 µg.
Figure 2 represents a typical PL pattern for pig muscle (longissimus dorsi). Further GC analysis of the PL fraction shows a rather broad spectrum of fatty acids with different carbon numbers and degrees of saturation (Fig.3). With the internal standard method, the exact amount of each fatty acid in the PL fraction can be measured.
After calculation of the average fatty acid molecular weight of the specific PL fraction, the total amount of the PL can be calculated by adding the molecular weight of the remaining basic structure to the according amount of fatty acid molecules. This method is already used in routine measurements in our laboratory, focusing on genotyp x environment interactions in German Landrace pigs.

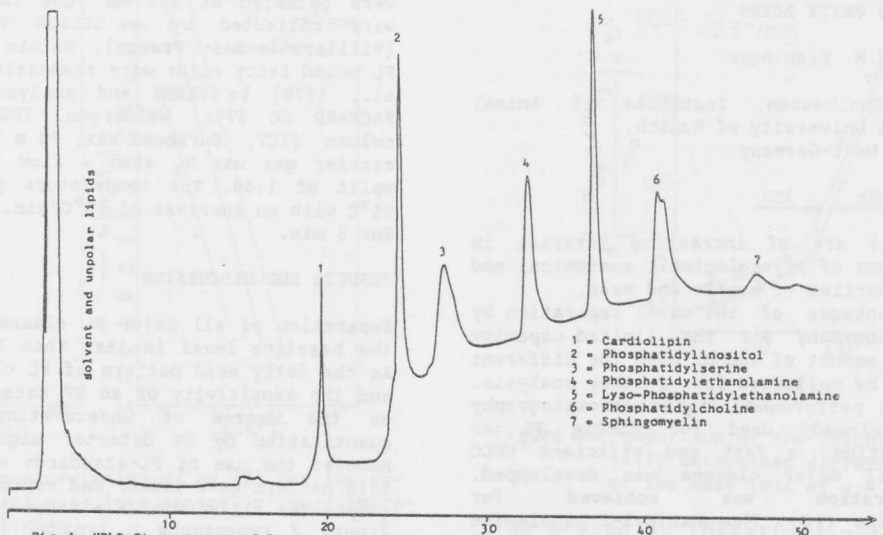


Fig. 1 HPLC-Chromatogram of Phospholipid Standards

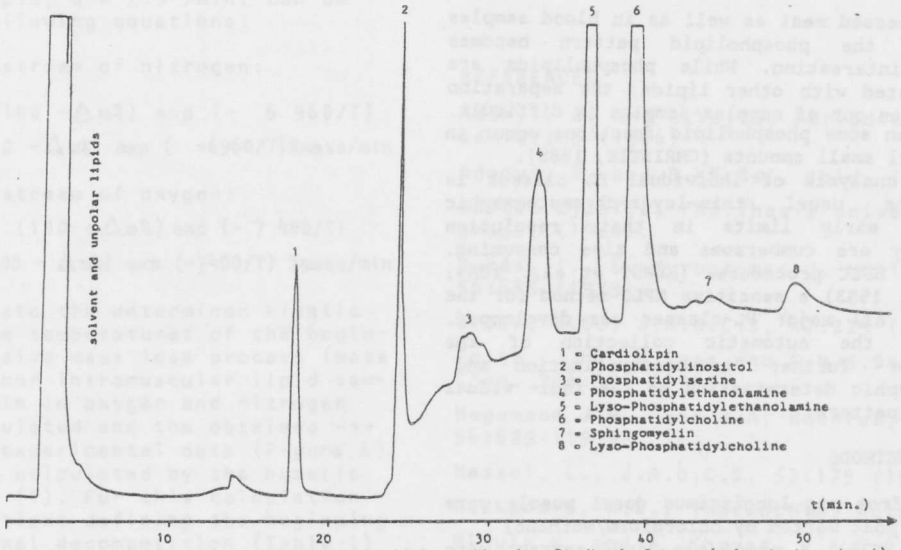


Fig. 2 HPLC-Chromatogram of a Phospholipid-Separation from Pig Muscle Extract (m.longissimus dorsi)

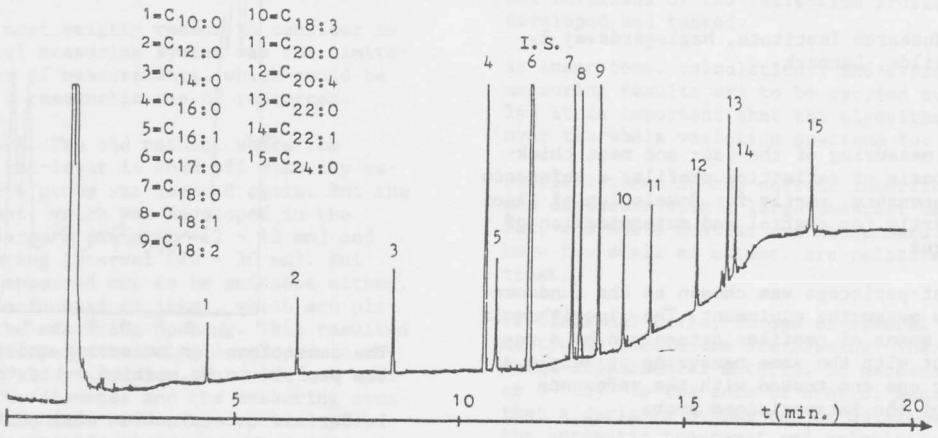


Fig.3 Gaschromatographic Separation of Fatty Acid Methyl Esters from the Phosphatidyl-Choline-Fraction of Pig Muscle (longissimus dorsi) After Transesterification

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