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Derivative enzymatic–spectrophotometric method for choline containing phospholipid determination in human serum, bile and amniotic fluid: recovery data by 'standard addition' method

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Abstract

Unpublished analytical data obtained by recovery tests by 'standard addition' method, concerning lecithin determinations in human serum, bile and amniotic fluid, using new derivative enzymatic–spectrophotometric method, recently pointed out by the authors, are reported and shortly discussed.

Keywords: Standard addition method; Human fluids; Choline containing phospholipid

1. Introduction

The analysis of phospholipids, important constituents of animal tissues, can be performed by several methods, which are however laborious, time consuming and not easily authomatized: real separation–extraction processes of the sample and derivatisation of the analyte are generally required using chromatographic methods [1,2]. Alternatively, the phospholipids concentration can be evaluated by total phosphorus analysis using spectrophotometric methods [3,4], however, these methods also require prior sample treatment using perchloric acid or perchloric acid–hydrogen peroxide mixture at about 180 °C. For this reason, a selective and direct enzymatic–spectrophotometric method was introduced to check choline containing phospholipids concentration[5], based on two in series reactions catalysed respectively by phospholipase D and choline oxidase enzymes, and on the subsequent reaction between hydrogen peroxide, obtained in the latter enzymatic reaction, and phenol and 4-aminophenazone in presence of peroxidase with the formation of red dye; the spectrophotometric measurement is performed at λ=500 nm. However, this method may present some drawbacks if the sample is pigmented or turbid. It was precisely to overcome these difficulties that the present authors are proposing a new method [6] based on the above two enzymatic reactions, using the first or second derivative in the enzymatic–spectrophotometric analysis, to directly determine the phospholipid (lecithin) content in human serum, amniotic fluid and bile samples, without the need for laborious sample pre-treatments.

In this short communication unpublished data, concerning several recovery tests, by standard addition method performed on sera, biles and amniotic fluids, have been reported, to evaluate better the accuracy of the method when applied to real samples of bioclinical interest.

2. Experimental

2.1. Apparatus

The spectrophotometric measurements were performed using a model 320 UV–VIS Perkin-Elmer double beam,

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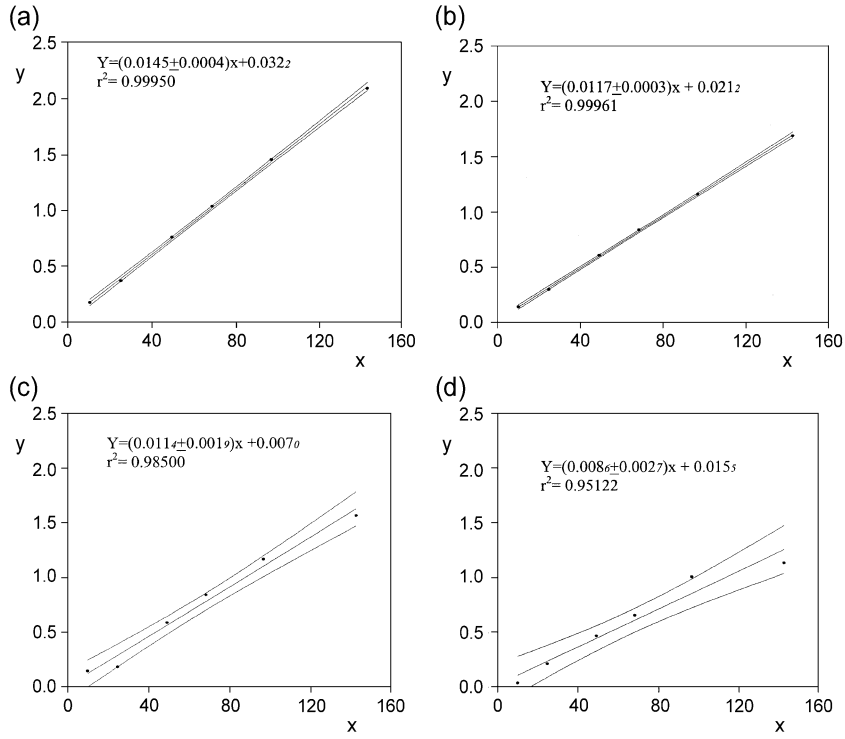

Fig. 1. (a) Equation, confidence interval and correlation coefficient of phospholipids calibration curve, obtained with 'standard method'. (y=absorbance; x=phosphatidilcholine concentration mg l^{-1}). (b) Equation, confidence interval and correlation coefficient of phospholipids calibration curve, obtained with 'first derivative method' ($y=\Delta l$ (a.u.); x=phosphatidilcholine concentration mg l^{-1}). (c) Equation, confidence interval and correlation coefficient of phospholipids calibration curve, obtained with 'second derivative' method (reading 412–500 nm) ($y=\Delta l$ (a.u.); x=phosphatidilcholine concentration mg l^{-1}). (d) Equation, confidence interval and correlation coefficient of phospholipids calibration curve, obtained with 'second derivative' method (reading 500–600 nm) ($y=\Delta l$ (a.u.); x=phosphatidilcholine concentration mg l^{-1}).

double grating monochromator spectrophotometer. The output signal was digitised, put through a central processor unit (CPU) and then sent to the recording system and/or interface to the Perkin-Elmer model 3600 data station. Using dedicated software, this system allowed both the spectrum scanning parameters to be programmed and the spectrophotometric data to be stored on disk for further processing. The spectrophotometer was maintained at 25±0.1 °C by means of a Colora ultrathermostat.

The spectrophotometer was able to convert the signal up to a fourth order derivative, with $\Delta\lambda$ ranging from 1 to 10. Quartz cuvettes with a 1.00 cm optical path length were used, together with a Julabo model UC 5 B thermostat.

Table 1
Repeatability of the human serum samples analysis

Measurement no.	'Standard method' (phosphatidylcholine in g l^{-1})	First derivative method (phosphatidylcholine in g l^{-1})	Second derivative method* (phosphatidylcholine in g l^{-1})	Second derivative method** (phosphatidylcholine in g l^{-1})
Serum (a)				
1	2.16	2.12	1.80	2.05
2	2.07	2.07	1.87	2.17
3	2.02	2.00	1.75	2.27
Average	2.08	2.06	1.81	2.16
s	0.07	0.06	0.06	0.11
Serum (b)				
1	2.81	2.81	2.48	2.65
2	2.81	2.83	2.52	2.48
3	2.83	2.86	2.62	2.60
Average	2.82	2.83	2.54	2.58
s	0.01	0.02	0.07	0.11

* Reading 412–500 nm.
** Reading 600–500 nm.