Analysis of Corticosteroids in Biological Fluids by High-Performance Liquid Chromatography with Fluorometric Detection

Key words: Cortisol—Corticosterone—Plasma—Saliva—HPLC

Analyses of cortisol and corticosterone have been used to assess pituitary-adrenal axis activity. At present, the measurement of these corticosteroids in biological materials is done mainly by radioimmunoassay. The specificity of this assay, however, is limited, because anti-cortisol antibodies, for example, cross-react to varying degrees with other steroids.

On the other hand, it is known that 11β-hydroxycorticosteroids fluoresce in strong sulfuric acid solutions, and can be measured by a fluorometer. Gotelli et al. and Shihabi et al. have used high-performance liquid chromatography (HPLC) to separate fluorescent derivatives, and have measured cortisol levels in serum and urine. Since the sulfuric acid-induced fluorescence is unstable, it is necessary to inject the reaction mixture into HPLC immediately after the formation of fluorescent derivatives is completed. The difficulty in timing the manual injection of the sample may be eliminated if an automated procedure is used for the sulfuric acid reaction and sampling in HPLC. For this purpose, we tried to develop a post-label HPLC method in which the steroids are first separated by HPLC and then changed to fluorescent compounds by an automated procedure.

Heparinized plasma (0.5 ml) or whole saliva (2 ml) was extracted with 15 ml

Fig. 1. Flow diagram for corticosteroid analysis by AutoAnalyzer.

SMC = single mixing coil
of dichloromethane, according to the method of De Moore et al. The organic phase was washed with 2 ml of 0.1 N sodium hydroxide and subsequently with 2.5 ml of water. Ten ml of the dichloromethane extract was evaporated to dryness under reduced pressure at room temperature. The residue was dissolved in 0.25 ml of 30% acetonitrile and injected to HPLC by means of an autosampler (Model KSST-120, Kyowa-Seimitu, Tokyo). For the separation of corticosteroids, the sample was eluted through a Zorbax CN column (DuPont, 5 μ, 4.6×250 mm) with 25% acetonitrile at 40°C. The flow rate was 0.5 ml/min and the effluent was continuously introduced into an AutoAnalyzer (Technicon, Chauncey) in which the separated corticosteroid of components were placed in contact with sulfuric acid and subsequently detected by fluorescence. The flow diagram is given in Fig. 1. Typical chromatograms of reference standards and a plasma extract are shown in Fig. 2. The peak height was proportional to the concentration of the steroids (Fig. 3). One picomole of cortisol or corticosterone is detectable by this method. Dexamethasone, 11α-hydroxyprogesterone and aldosterone do not show any detectable fluorescence in this method, but prednisolone, 21-deoxycortisol and 11β-hydroxyprogesterone are positive. It should be noted that prednisolone interferes with the analysis of cortisol, because the retention times of the two

![Fig. 2. Chromatograms of reference standards (upper) and a plasma extract (lower).](image-url)
Fig. 3. Calibration curves for cortisol and corticosterone.

Fig. 4. Relationship between plasma cortisol level and saliva cortisol level in 9 young adults.
steroids in HPLC are almost the same.

The recovery of cortisol and corticosterone throughout the procedure was about 90%, and the coefficient of variation of 5 cortisol measurements was about 5%.

Blood was drawn from the antecubital vein of 9 healthy young adults (18–23 years old, 5 males and 4 females) at 10 a.m. Whole saliva was also obtained from the same subjects. The mean cortisol level of the subjects was 239 pmol/ml in plasma and 4.35 pmol/ml in saliva. The values are comparable to those cited in the literature. The correlation coefficient between the cortisol levels in plasma and saliva was highly significant (p<0.01, Fig. 4), consistent with the finding of Katz and Shannon.

The HPLC method described above has the advantage of avoiding the use of radioisotopes. The method is simple, sensitive and specific, so that it is suitable for the measurement of plasma and saliva cortisol in routine laboratory analysis. This method would be extended to the measurement of urinary cortisol.

REFERENCES


National Institute of Industrial Health
21-1, Nagao 6-chome, Tama-ku,
Kawasaki, 214, Japan

(Received August 25, 1988 and in revised form September 16, 1988)