



Analysis of Glucocorticoids by HPLC

Udo Huber

Pharmaceutical

Glucocorticoids or corticosteroids are steroid hormones synthesized in the adrenal glands. They have different functions in the human body, such as regulation of sugar, protein and mineral circulation, regulation of transcription of certain genes, and so on.

Figures 1 and 2 show the separation of the glucocorticoids beclomethasone, hydrocortisone and hydrocortisone acetate and prednisolone, prednisolone acetate, betamethasone and betamethasone valerate using gradient analysis on a reversed phase column and UV detection. The autosampler temperature was set to 4°C to avoid decomposition of the samples.

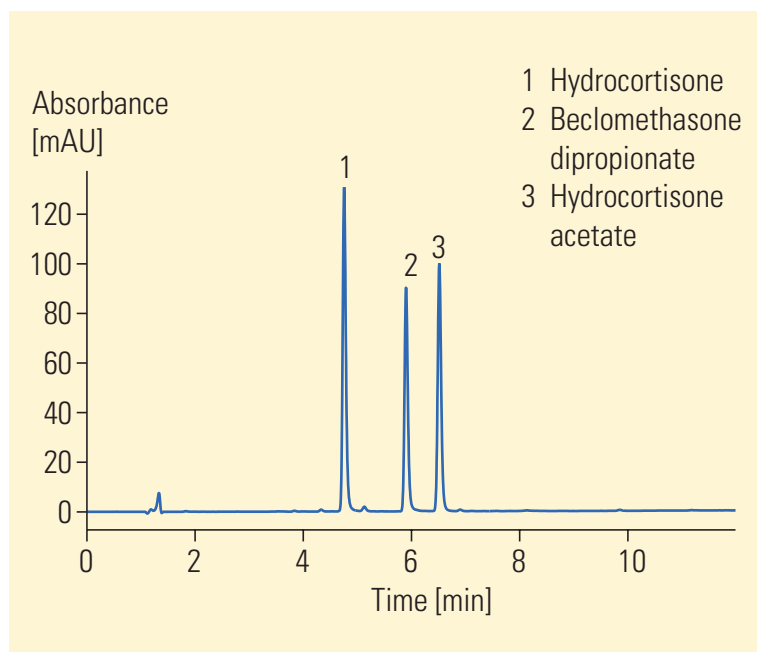


Figure 1
Analysis of three glucocorticoids

Conditions

Column

4 x 125 mm Hypersil ODS, 5 µm

Mobile phase

A = water, B = acetonitrile

Flow rate

1.0 ml/min

Gradient

at 0 min 20 % B

at 10 min 80 % B

Column wash

at 12 min 20 % B

UV detector

variable wavelength detector

254 nm, standard cell

Column compartment temperature

25 °C

Stop time

12 min

Post time

5 min

Injection volume

5 µl



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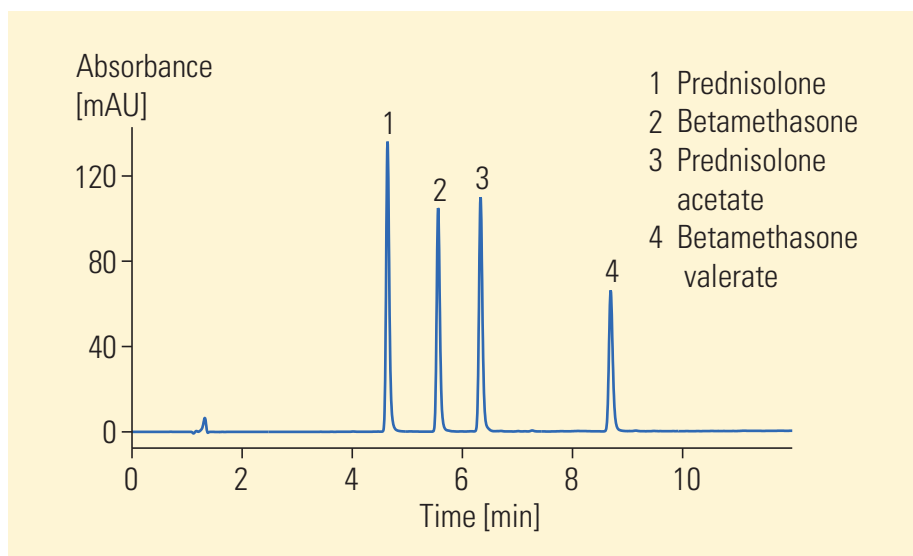


Figure 2
Analysis of four glucocorticoids

HPLC Performance

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs (100 mg/l))*	Precision of Area (RSD of 10 runs) (100 mg/l)*	Linearity Correlation factor-(0.1-100 mg/l)*
Hydrocortisone	0.1	0.03	0.60	0.99998
Beclomethasone	0.1	0.03	0.53	0.99997
Hydrocortisone Acetat	0.1	0.03	0.42	0.99998
Prednisolone	0.1	0.03	0.15	0.99998
Betamethasone	0.1	0.03	0.25	0.99998
Prednisolone Acetat	0.1	0.02	0.29	0.99998
Betamethasone Valerate	0.1	0.01	0.33	0.99999

* Injection volume: 5 µl

The performance of the HPLC method is shown in the table above.

The HPLC method presented here shows an easy but reliable and precise analysis of the glucocorticoids beclomethasone, hydrocortisone and hydrocortisone acetate, prednisolone, prednisolone acetate, betamethasone and betamethasone valerate. The values for LOD, precision of RT, precision of area and linearity show the good performance of the analysis.

Equipment

Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell 10-mm path length, 13-µl cell volume

Alternative:

- Binary pump
- Vacuum degasser
- Diode array detector standard flow cell 10-mm path length, 13-µl cell volume
- Agilent ChemStation + 3D software

Columns

- Hypersil ODS µm, 4 x 125 mm (Agilent part number 792618-564)
- Recommended: Guard cartridges Hypersil ODS, 5 µm, 4 x 4 mm (Agilent part number 7992618-504, 10/pk)

Note:

Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler.

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