HPLC ANALYSIS OF NINE CORTICOSTEROIDS IN "NATURAL CREAMS" FOR ATOPIC ECZEMA

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The aim of the study was to determine whether "natural creams" sold for treatment of childhood atopic eczema illegally contain corticosteroids with a newly developed rapid and simple HPLC analysis with UV detection. HPLC analysis was performed using a Schimadzu LC-2010 chromatographic system (Schimadzu, Kyoto, Japan) consisting of a LC-20AT Prominence liquid chromatograph pump with DGU-20A5 Prominence degasser, a SPD-M20A Prominence Diode Array Detector, and a SIL-20 AC Prominence auto sampler. Data analyses were done using Class VP 7.3 Software. The elution was carried out on a column Purospher STAR® RP 18e (250 x 4.6 mm i.d., particle size 5µ m), with a mobile phase consisted of acetonitrile and water in a gradient mode, at a flow rate of 1.0 mL min^{-1}, at controlled column temperature (25°C). Detection of nine different corticosteroids (dexamethasone, prednisolone, methylprednisolone, fluocortolone, hydrocortisone, mometasone, betamethasone, beclomethasone, and diflucortolone) was carried out with DAD detector at a wavelength of 240 nm. The injection volume was 10 µl. The samples were five different creams sold for the treatment of childhood atopic eczema (marketed as steroid free) and were submitted by the state regulatory authority, Bureau of the medicines. The method was fully validated according to the ICH (International Conference on Harmonization) guidelines by the determination of linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). Selectivity of the method was proved with the chromatographic peak resolution obtained between each of the nine different corticosteroids and the characteristic UV spectra. Linearity of the method was tested in the range of 0.4 – 8 µg mL^{-1} for all substances analysed. Experimental data showed high level of linearity for all corticosteroids (ranging from $R^2 = 0.9981$ for diflucortolone to $R^2 = 1.0$ for dexamethasone, prednisolone, fluocortolone, hydrocortisone, mometason, and beclomethasone). The accuracy of the method was demonstrated by the values obtained from the recovery experiments (ranging from 98.67%, for diflucortolone to 101.33% for beclomethasone). The method was successfully applied to the analysis of real samples of creams sold as steroid free. The analyses revealed that two of the samples contained corticosteroids. The proposed HPLC method allows a simple, accurate, and rapid identification of corticosteroids in creams used for the treatment of atopic eczema.

Table 1. LOD and LOQ of the method

Corticosteroid	RT (min)[1]	LOD (ng)[2]	LOQ (ng)[3]
Dexamethasone	5.20	0.18	0.56
Prednisolone	6.81	0.20	0.59
Methylprednisolone	7.44	0.20	0.60
Fluocortolone	9.91	0.42	1.29
Hydrocortisone	10.58	0.23	0.69
Mometasone	23.13	0.22	0.66
Betamethasone	23.56	0.36	1.09
Beclomethasone	25.64	0.46	1.40
Diflucortolone	26.56	0.78	2.36

[1] Retention Time
[2] Limit of Detection
[3] Limit of Quantification

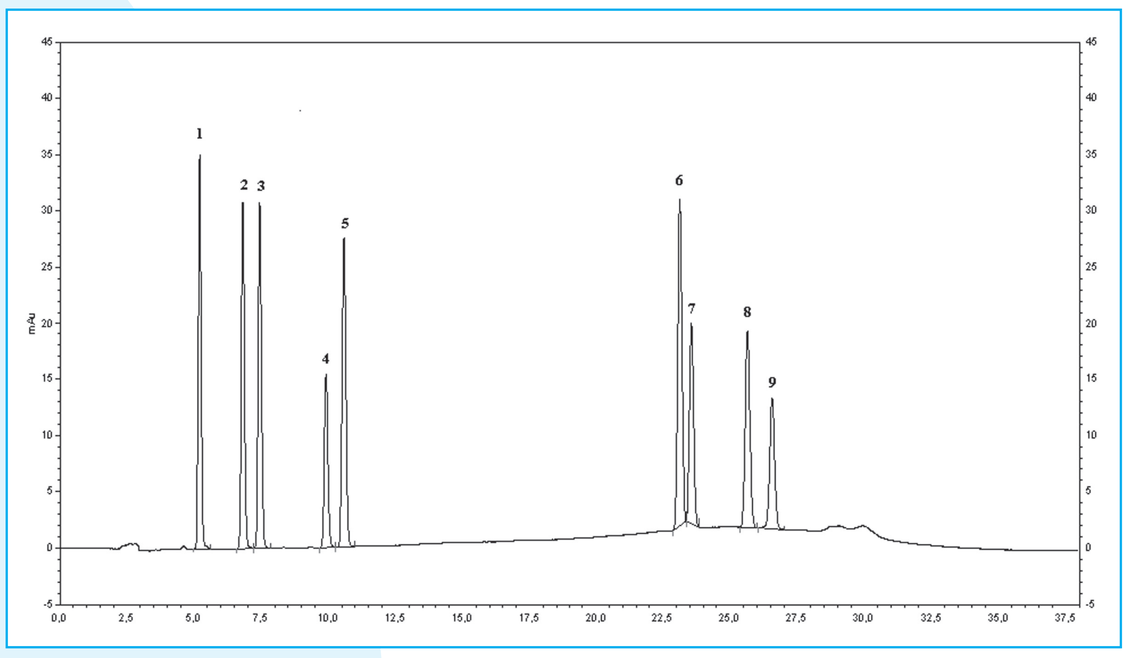

Figure 1. A typical chromatogram of the mixed standard solution containing: dexamethasone (1), prednisolone (2), methylprednisolone (3), fluocortolone (4), hydrocortisone (5), mometasone (6), betamethasone (7), beclomethasone (8), and diflucortolone (9).

Literature Reference

1. ICH Q2R1: Validation of Analytical Procedures: Text and Methodology, Proceeding of the International Conference on Harmonization of technical Requirements for Registration of pharmaceuticals for Human Use, Geneva, Switzerland, 1996.
2. Ramsay HM, Goddard W, Gill S, Moss C. Herbal creams used for atopic eczema in Birmingham, UK illegally contains potent corticosteroids. Arch Dis Child. 2003 Dec;88(12): 1056-7. PubMed PMID: 14670768; PubMed Central PMCID: PMC1719403.