Extraction of Corticosteroids from Plasma using ISOLUTE® SLE+ Supported Liquid Extraction Plates

Application Note

Introduction

This Application Note highlights the use of ISOLUTE SLE+ supported-liquid extraction plates for the extraction of corticosteroids from human plasma.

Supported-liquid extraction (SLE) is a 96-well sample preparation technique that is analogous to traditional liquid-liquid extraction (LLE). The extraction interface occurs between the buffered sample absorbed onto an inert solid support and a water immiscible solvent (see Figure 1). This provides excellent extraction efficiency while alleviating many of the liquid handling and emulsion formation issues associated with traditional LLE.

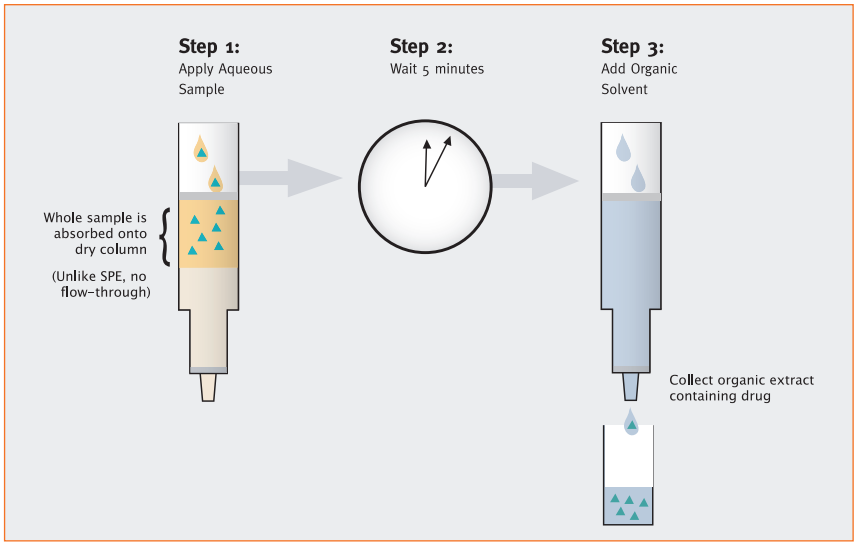


Figure 1. The supported-liquid extraction process using the ISOLUTE SLE+ supported-liquid extraction plate (single well shown).

Method

Reagents

All analytes (see Table 2) and formic acid were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK).

Supported Liquid Extraction Procedure

Plate:	ISOLUTE SLE+ 200 mg supported-liquid extraction plate (part number 820-0200-P01)
Sample:	Blank human plasma (100 µL) was spiked with the corticosteroids at 200 ng/mL. The plasma was then diluted 1:1 v/v with water prior to loading. This sample dilution results in approximate loading pH of 8.0.
Sample Application:	The pretreated plasma was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.
Analyte Extraction:	Ethyl acetate (1 x 1 mL). Allow solvent to flow for 5 minutes under gravity. Apply vacuum (-15 "Hg / -0.5 bar) for 2 minutes to complete elution.
Post Extraction:	The extracts were evaporated to dryness and the analytes reconstituted in 500 µL of 80:20 (v/v) H_2O/MeOH prior to analysis.

Biotage

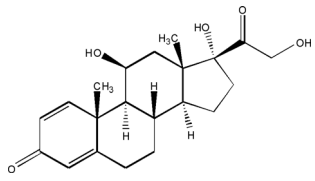
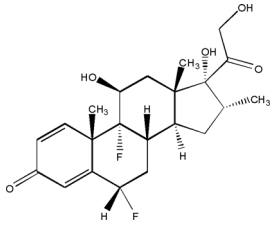
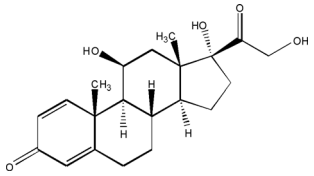
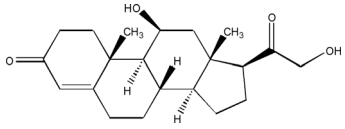
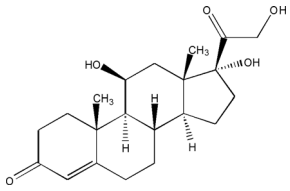
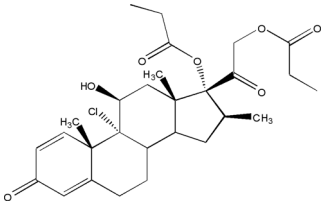
HPLC Conditons

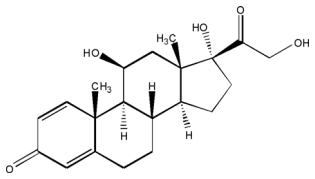
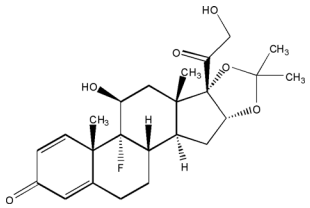
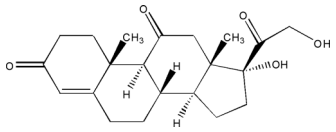
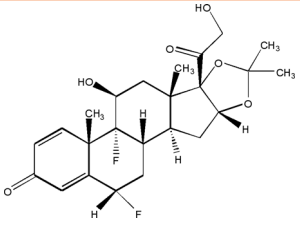
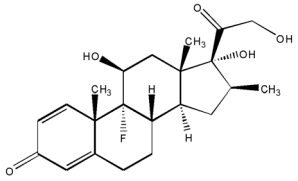
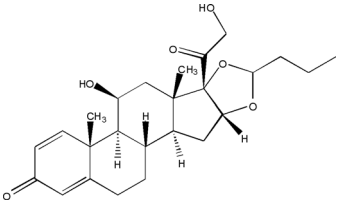
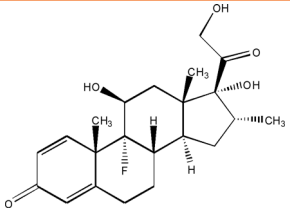
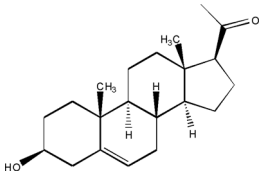
Instrument:	Waters® 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column:	Zorbax® Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id, 3.5 µm) (Agilent Technologies, Berkshire, UK).
Guard Column:	C8 guard column (Agilent Technologies, Berkshire, UK).
Mobile Phase:	0.1% formic acid aq and MeCN (acetonitrile) at a flow rate of 0.25 mL/min.
Gradient:	See **Table 1** below.
Injection Volume:	10 µL
Temperature:	Ambient

Table 1. HPLC Gradient Conditions

Time	0.1% Formic acid aw (%)	MeCM (%)
0	80	20
15	60	40
15.1	10	90
19	10	90
19.1	80	20

Table 2. Corticosteroid structures

Analyte	Structure	Analyte	Structure
Triamcinolone		Flumethasone	
Prednisolone		Corticosterone	
Hydrocortisone		Beclomethasone	

Analyte	Structure	Analyte	Structure
Prednisone		Triamcinolone acetonide	
Cortisone		Fluocinolone Acetonide	
Betamethasone		Budesonide*	
Dexamethasone		5-Pregnen-3β-ol-20-one	

*Two structural isomers observed.

Results

Using the procedure described, recoveries for the corticosteroids is shown in Table 3 / Figure 2.

Analyte	Recovery	RSDs
Triamcinolone	93	4
Prednisolone	93	1
Hydrocortisone	98	2
Prednisone	95	1
Cortisone	96	2
Betamethasone	92	2
Dexamethasone	92	2
Flumethasone	91	2
Corticosterone	94	2
Beclomethasone	91	2
Triamcinolone Acetonide	91	3
Fluocinolone Acetonide	90	3
Budesonide Structural Isomer 1	87	3
Budesonide Structural Isomer 2	89	2
5-pregnen-3β-ol-20-one	95	4

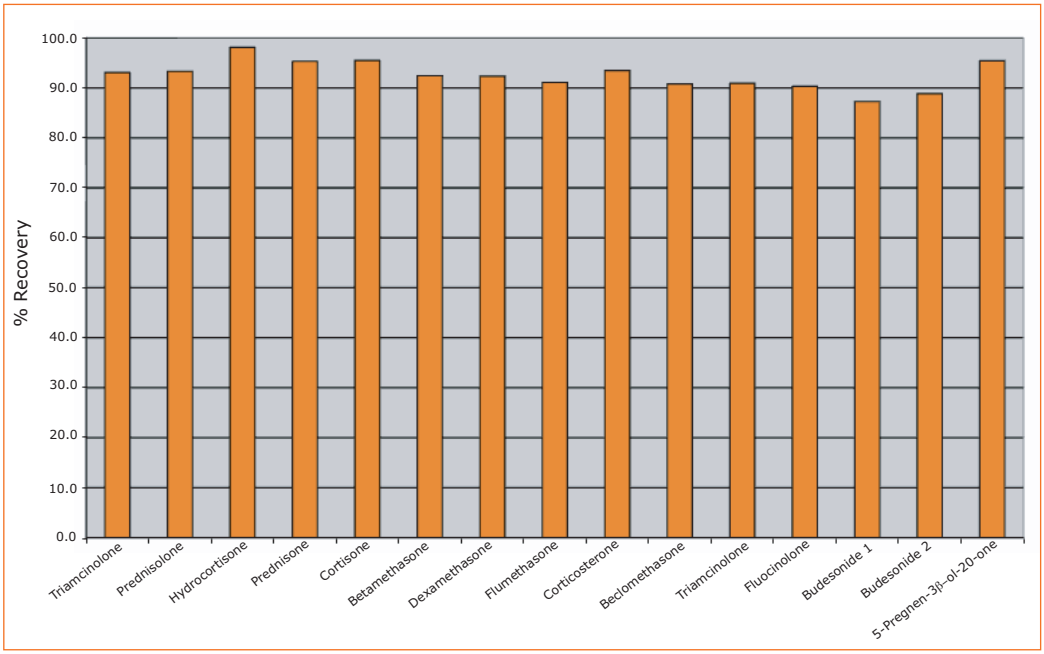


Figure 2. Corticosteroid recoveries

Conclusions

ISOLUTE SLE+ supported-liquid extraction plates can be used to extract a range of corticosteroids from human plasma with quantitative recoveries and RSDs < 10%.

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