Tuning of a MISPE procedure for the simultaneous extraction of Dexamethasone and Betamethasone

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ABSTRACT

A molecularly imprinted solid-phase extraction (MISPE) procedure based on precipitation polymerization was developed for the simultaneous extraction of dexamethasone (DM) and its structural isomer, betamethasone (BM), from biological matrices. A DM-imprinted polymer was synthetized using methacrylic acid as monomer and divinylbenzene as cross-linker, and the porogen was a mixture of acetonitrile and toluene (3:1). After removal of the template, loading, washing and elution steps were optimized. The maximun recovery of DM and BM was achieved when loading with toluene and washing with 5% acetonitrile in toluene, and eluting with 1% acetic acid in methanol.

INTRODUCTION

■ Corticosteroids, such as dexamethasone (DM) and betamethasone (BM), are synthetic glucocorticoids which are frecuently employed as antipyretic, anti-inflammatory, anti-allergy drugs and as growth-promoting agents in cattle [1]. All synthetic corticoids were banned for fattening purposed by Council Directive 96/22/EC [2]. However, due to the possible occurrence of cross contamination into feed industry, a recent maximum residue limit (MRL) has been established for DM at 2 µg/kg in liver, 0.75 µg/kg in kidney and muscle and 0.3 µg/kg in milk [3]. Therefore, control appears necessary to survey the misuse of these corticoids in food producing animals and to assure food safety.

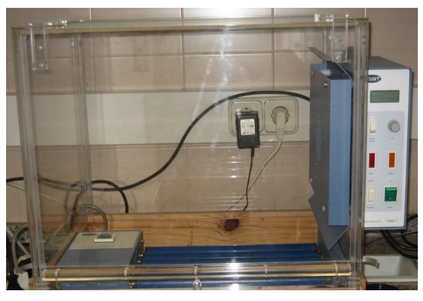
■ Solid-phase extraction (SPE) has frequently been employed for sample cleaning-up due to its great simplicity, low cost and easy automation. However, most of the times commercial cartridges lack specificity and possibilities of reuse, giving lower recoveries than molecularly imprinted solid phase extractions (MISPE) [4]. Consequently, molecularly imprinted polymers (MIPs) are being introduced in several analytical fields as chromatography, among others, to obtain a higher degree of selectivity during analysis. A methacrylic acid-based polymer was prepared by precipitation polymerization using DM as a template and a mixture acetonitrile:toluene (3:1) as porogen solvent. After removal of the template by Soxhlet's extraction, the optimal loading, washing and elution conditions for MISPE of DM and BM were selected.

EXPERIMENTAL PROCEDURE

1. Polymerization mixture

Template	Dexamethasone	1mmol
Monomer	Methacrylic acid	6mmol
Cross-linker	Divinylbenzene	20mmol
Initiator	2,2'-azobis (2-methylbutyronitrile)	0.24mmol
Porogen	Acetonitrile 75% (ACN) Toluene 25% (TOL)	12.5ml

2. Precipitation polymerization



Incubator S160D & Roller mixer SRT1 (Stuart Scientific, UK) 24h-60º

3. Filtration and washing

ACN & MeOH

4. Template removal

Soxhlet extraction MeOH/AcOH 1:1→18h

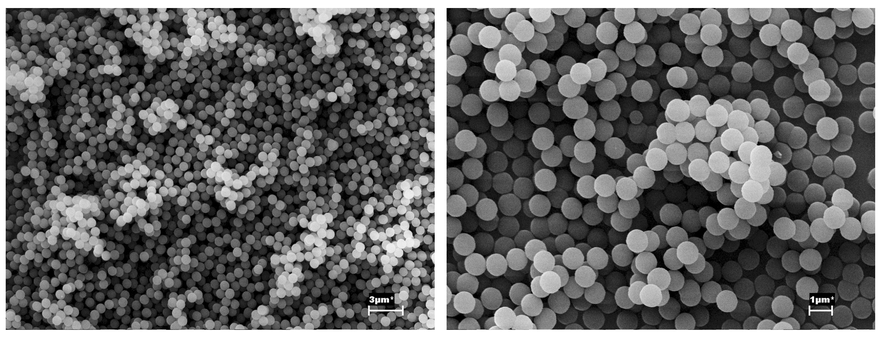
5. Washing

MeOH & ACN

6. Dry, weigth and characterization of MIP and NIP (SEM)

➢The yield of the polymerization procedure:

MIP →0.42 g (83.2%) NIP →0.37 g (75%)

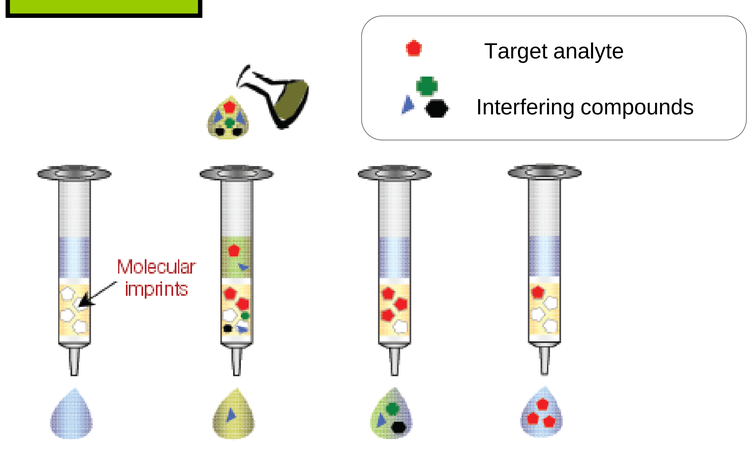


* The whole process has been applied to MIP and NIP simultaneously.

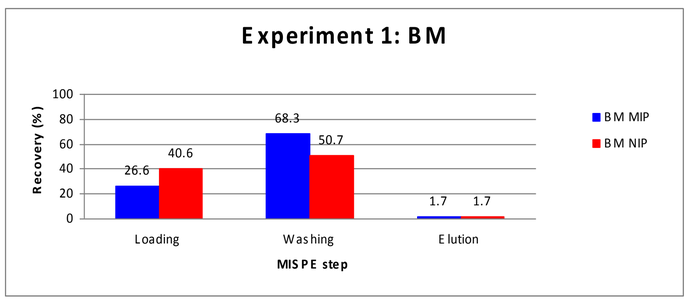
7. Cartridges

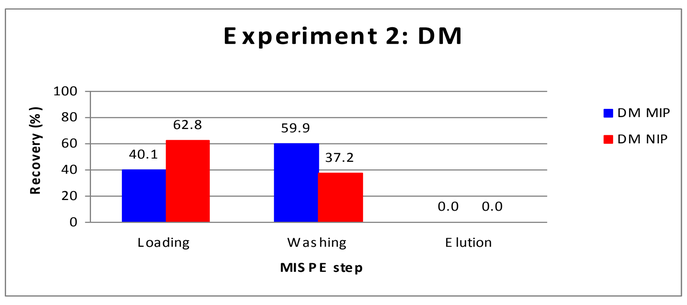
0.05g of polymer (MIP, NIP)

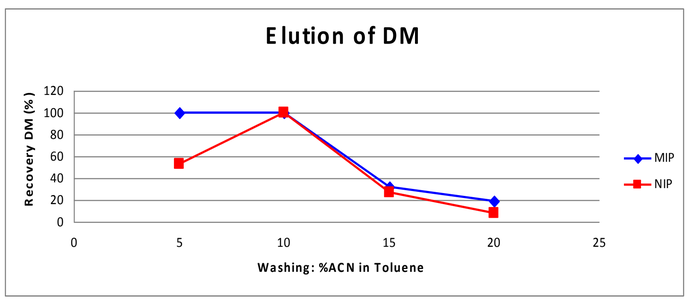
8. MISPE



RESULTS AND DISCUSSION







MISPE step	Experiment 1	Experiment 2
Conditioning	ACN	TOL
Loading	ACN	TOL
Washing	ACN	TOL:ACN
Elution	MeOH:HOAc (99:1)	MeOH:HOAc (99:1)

➢Concerning loading solution, first experiments were made using ACN, as it was the principal porogen. However, results were not promising since a little or no analyte was retained until elution step. Toluene was also evaluated for sample loading and washing (with different percentages of ACN). The retention was improved in the second experiment, obteining the best recoveries for DM when washing with toluene 5% ACN, because the difference between MIP and NIP was considerable.

REFERENCES

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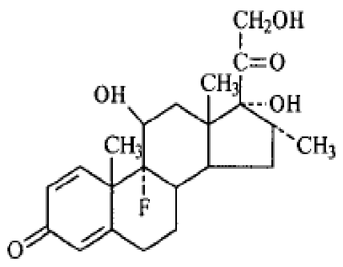
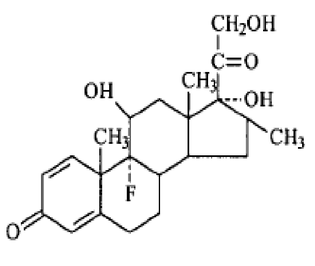
Dexamethasone





Betamethasone