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# A rapid determination of acidic pharmaceuticals in environmental waters by molecularly imprinted solid-phase extraction coupled to tandem mass spectrometry without chromatography

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ABSTRACT

This study presents a rapid analytical method that involves an off-line molecularly imprinted solid-phase extraction (MISPE) specific for non-steroidal anti-inflammatory drugs (NSAIDs) as a selective sample pretreatment coupled directly to tandem mass spectrometry (MS/MS). The developed methodology provided sensitive and selective detection and quantification of six acidic pharmaceuticals in wastewaters without the chromatographic separation.

The optimised MISPE procedure enabled to extract effectively the studied analytes from effluent and influent wastewaters with satisfactory recovery values (from 62% to 103%).

The analytical method developed was validated using 50 mL of effluent wastewaters, obtaining limits of detection (LODs) lower than 0.1 $\mu g\ L^{-1}$ for all the compounds studied. The method was successfully applied for the determination of these acidic pharmaceuticals in effluent and influent wastewaters. The analytes and their concentration are in line with other studies in which these analytes are determined by SPE–LC–MS/MS in similar samples.



## 1. Introduction

Nowadays, rapid analytical methods are required in order to analyse the maximum number of samples in the minimum time period. Up to now, many analytical methods have been developed to determine acidic pharmaceuticals, such as non-steroidal anti-inflammatory drugs (NSAIDs), among others, in complex matrices, mainly using solid-phase extraction (SPE) followed by liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) [1–4]. However, these methodologies usually involve time-consuming procedures from the sample collection right through to their quantification. In order to reduce analysis time, it should be worthy to improve on sample pretreatments and couple them directly to a specific and sensitive detection system, without chromatographic analysis.

MS or tandem MS is currently the most commonly used detection technique for the identification and quantification of pharmaceuticals in complex matrices due to its high sensitivity, selectivity and speed [5]. Despite its numerous advantages, this technique using an electrospray ionisation (ESI) source may suffer from ion suppression/enhancement caused by interferences present in complex matrices [6]. For this reason, removing as much as possible interfering matrix compounds in order to minimise these matrix effects is a challenge.

In recent years, few studies have reported the direct coupling of an extraction technique to a detection technique. For instance, the on-line SPE–MS system has been applied for the determination of clenbuterol in urine [7,8] and prednisolone in serum [9], while the coupling between SPE and MS/MS enabled to determine antihypertensive drugs in human plasma and urine [10]. These studies have been developed using non-selective SPE sorbents, whose protocols did not include an effective clean-up step, and many matrix compounds were still present in the SPE eluate, obtaining higher limits of detection (LODs) than expected.

To tackle this problem, it is necessary to purify the samples as much as possible in order to eliminate interferences. Molecularly imprinted solid-phase extraction (MISPE) has been defined as a selective extraction technique because of its molecular-recognition technology, which allows specific binding between the target molecule or template and the polymer structure [11,12]. Currently, new approaches are being developed in this field which apply these selective molecularly imprinted polymers (MIPs) coupled directly to detection techniques in order to eliminate as many of the interferences as possible without the losses of target analytes.

On this point, a few studies have been reported using MISPE–MS, such as for the determination of fluoroquinolones in urine [13], benzodiazepines in human plasma [14] and phenothiazines in urine [15], as well as another that used a MISPE-fluorescence detector (FD) to determine ochratoxin A in wheat samples [16]. These studies

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emphasised the selectivity and simplicity of the methodology in comparison with the classical methods which included SPE followed by LC prior to MS/MS for the determination of different drugs in environmental and biological matrices [17–23]. However, to the best of our knowledge, MISPE has never been coupled directly to MS/MS, which might significantly improve the sensitivity and selectivity of the methodology.

In view of this, the aim of the present work is to develop a rapid and selective analytical method for the determination of six acidic pharmaceuticals in wastewaters by MISPE–MS/MS.

## 2. Materials and methods

### 2.1. Materials

Clofibric acid, naproxen, ibuprofen, fenoprofen, diclofenac and gemfibrozil were purchased from Sigma-Aldrich (Steinheim, Germany). All pharmaceutical standards used were of high purity grade (>97%). As internal standard (IS), gemfibrozil-$d_6$ (98%) (100 mg $L^{-1}$ in dioxane) from Cambridge Isotope Laboratories (Andover, USA) was used.

Stock solutions of individual standards were prepared by dissolving each compound in methanol (MeOH) at a concentration of 1000 mg $L^{-1}$. A mixture of all compounds in MeOH at a concentration of 50 mg $L^{-1}$ was prepared weekly. Working solutions were prepared daily from these stock solutions diluted in MeOH/$H_2O$ at pH 7 (60:40, v/v). These solutions were stored at 4 °C. The structures and p$K_a$ values of these substances are presented in Table 1.

HPLC grade MeOH and acetonitrile (ACN) were purchased from SDS (Peypin, France). Ultrapure water was obtained from a water purification system (Veolia, Sant Cugat del Vallès, Spain) and nitrogen ($N_2$) (99%) was supplied by Carburos Metálicos (Tarragona, Spain). Acetic acid ($CH_3COOH$) (≥99.8%) from SDS (Peypin, France), hydrochloric acid (HCl) (37%) from Prolabo (Bois, France) and ammonium hydroxide ($NH_4OH$) (25%) from Panreac (Barcelona, Spain) were used to adjust the pH of the carrier liquid and the samples.

### 2.2. Sample collection

The wastewater samples were collected from the influent and effluent of two domestic sewage treatment plants (STPs), which are located in two cities with populations of around 120,000 habitants each, by using pre-cleaned amber glass bottles. All the samples were filtered using a 0.45 μm nylon membrane (Supelco, Bellefonte, PA, USA), acidified to pH 3 (HCl) and stored at 4 °C until analysis.

### 2.3. Molecularly imprinted solid-phase extraction

150 mg of a commercially available MIP, namely Affinilute MIP-NSAIDs (Biotage, Barcelona, Spain), were packed manually and placed into 6 mL polyethylene cartridge with 2 polypropylene frits (~10 μm) (Symta, Madrid, Spain). The cartridges were placed in an SPE manifold (Teknokroma, Barcelona, Spain) and connected to a vacuum pump. They were conditioned with 5 mL of ACN, 5 mL of MeOH and 5 mL of $H_2O$ adjusted to pH 3. The samples adjusted to pH 3 were loaded through the MIP. A clean-up step was then performed with 5 mL of ACN:$H_2O$ (40:60, v/v). In order to elute the retained analytes, 10 mL of MeOH:acetone (80:20, v/v) with 1% $CH_3COOH$ was passed through the cartridge. Elution extracts were evaporated to dryness under a gentle flow of $N_2$. Before MS/MS injection, the elution fractions were reconstituted to a final volume of 1 mL of MeOH/$H_2O$ at pH 7 (60:40, v/v), to which gemfibrozil-$d_6$ (IS) was added at 50 μg $L^{-1}$, in order to correct LC injection and ionisation variability.

### 2.4. Instrumentation

All extracts were injected by flow injection analysis (FIA) using an Agilent quaternary pump 1200 series and an automatic injector (the volume injected was 50 μL) connected to a 6410 series triple quadrupole mass spectrometer using ESI from Agilent Technologies (Waldbronn, Germany).

The optimised carrier liquid, used to push the extracts from the injector to MS/MS, was composed of MeOH/$H_2O$ at pH 7 (60:40, v/v). The flow rate was set at 0.8 mL $min^{-1}$.

With respect to MS/MS detection, $N_2$ was used as the collision gas and its flow rate was set at 12 L $min^{-1}$. A source temperature of 300 °C, a nebuliser pressure of 40 psi ($N_2$) and a capillary potential of 4000 V were applied. Multiple reaction monitoring (MRM) in negative ionisation mode was used to determine all analytes. Table 1 details MRM transitions, cone voltage and collision energy for each compound.

## 3. Results and discussion

### 3.1. MS/MS conditions

The different MS/MS parameters were adjusted, injecting each compound at 250 μg $L^{-1}$ individually by FIA. Table 1 shows the optimum MS/MS conditions for each analyte in negative ESI. It was possible to obtain two different MRM transitions (selected as quantifier and qualifier) for all target analytes, except for ibuprofen, the MS/MS spectrum of which only contained one diagnostic ion and, hence, only one MRM transition was achieved for this compound. However, this compound was not initially excluded from the study due to its high prevalence in environmental water samples at high concentration levels [23]. Moreover, we injected a mixture of all the analytes and we checked that the same response was obtained for each analyte, rather than injecting them individually by FIA mode. Thus, it means that the signal of each analyte did not interfere with the signal of the rest of analytes. Therefore, MS/MS can be considered selective for the studied compounds under these conditions.

Next, the composition of the carrier liquid was optimised in order to enhance the analyte response. In case that the analytes were first separated using LC and then detected by MS/MS, the mobile phase had to be selected to obtain both a successful separation and proper ionisation of the compounds. However, when working with the direct coupling MISPE–MS/MS, the only requirement of the carrier liquid composition is to achieve the best solvent for ionisation in ESI interface. With this in mind, different solutions of MeOH or ACN (as organic solvent) combined with acidic or basic water were tested as the carrier liquid. To be specific, the carrier liquid compositions were: MeOH/$H_2O$ at pH 3 (80:20, v/v), ACN/$H_2O$ at pH 3 (80:20, v/v), MeOH/$H_2O$ at pH 7 (80:20, v/v), ACN/$H_2O$ at pH 7 (80:20, v/v), MeOH/$H_2O$ at pH 7 (60:40, v/v) and ACN/$H_2O$ at pH 7 (60:40, v/v). It should be mentioned that, in all instances, the injected solution containing the analytes and IS were prepared in the same composition as the carrier liquid. Fig. 1 shows the response achieved for all the analytes studied with the different carrier liquids tested.

First of all, MeOH/$H_2O$ at pH 3 (80:20, v/v) and ACN/$H_2O$ at pH 3 (80:20, v/v) were tested. These are typical mobile phases applied in LC since at pH 3 these analytes are in the neutral form, which would be appropriate for separation along the LC column. Nevertheless, solutions at pH 3 are not the most suitable for promoting the ionisation in the negative ESI interface, as shown in Fig. 1, in which the lowest areas were obtained. When the aqueous phase was adjusted to pH 7, maintaining the composition of the carrier liquid (80:20, v/v) and in both MeOH and ACN, the response increased for all target analytes. This fact could be explained because, under these