

Determination of Fenspiride Hydrochloride Residues on Pharmaceutical Manufacturing Equipment Surfaces by HPLC Method

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The cleaning procedure must be validated, so special attention must be devoted to the methods used for determination of trace amounts of drugs. A rapid, sensitive, and specific reverse phase high-performance liquid chromatographic (HPLC) method was developed for the quantitative determination of fenspiride hydrochloride residues on pharmaceutical manufacturing equipment surfaces. The calibration curve was linear over a concentration range from 1.0 to 100.0 µg/ml with a correlation coefficient of 0.99994. The detection limit and quantitation limit were 0.41 µg/ml and 1.25 µg/ml, respectively. The developed method was validated with respect to specificity, linearity, limit of detection, accuracy and precision.

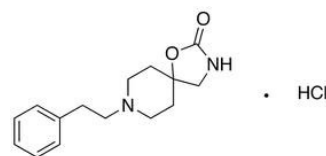
Introduction

In pharmaceutical industries, it is very important step in the manufacture of pharmaceutical products is the cleaning of equipment and surfaces. The cleaning procedure must be validated, so special attention must be devoted to the methods used for analysis of trace amounts of drugs.

This is necessary not only to ensure the quality of the next batch of different products but also to prevent cross-contamination; it is also a World Health Organisation good manufacturing practice (GMP) requirement [1]. There are two main types of sampling that have been found acceptable: The most desirable direct surface sampling of the equipment by using swabs and the use of the final rinse solution.

Typical acceptance criteria used in the pharmaceutical industry are the 1/1000 dosage criteria (1/1000 reduction of the lowest therapeutic dose of the previous drug product), the 10 ppm criteria (no more than 10 ppm of any active pharmaceutical ingredient will appear in the next product), or the visual clean criteria (no residue will be visible on the equipment after the cleaning process). Several mathematical formulas were proposed to establish the acceptable residual limit [2].

Fenspiride hydrochloride (FH, 8-(2-phenylethyl)-1-oxo-3,8-diazaspiro[4,5]decan-2-one hydrochloride) is an effective drug in case of inflammatory diseases of otolaryngological system and respiratory tracts. It is also used as a complex therapy for bronchial asthma, allergic rhinitis. Its structural formula is as follow:



Fenspiride hydrochloride (FH)

In the 50 last years only several methods for determination of FH in pharmaceutical preparations and biological samples (human plasma and urine, horse body fluids) have been described. The first method for the quantification of fenspiride was described in 1976 (spectrophotometric determination) [3].

The method for the quantification of fenspiride in plasma and urine which was described in article [4] included liquid-liquid extraction of analyte from the biomatrix. The quantification of fenspiride was run by HPLC method on the reversed-phase column, with electrochemical detection and ultraviolet detection. In the paper [5] fenspiride and its metabolites in urine were detected with the help of capillary gas chromatography-mass spectrometry method.

The selective LC-MS/MS method was developed for more than 250 basic drugs screening including fenspiride in the supernatant of enzyme hydrolysed equine urine after extraction on the short Oasis HLB® column [6].

One more method based on gas chromatography-mass spectrometry was developed for narcotics and stimulants in equine urine screening [7].

In the article [8] fenspiride was determined in human plasma using the liquid-liquid extraction of fenspiride and the internal standard in 1-octanol, followed by direct injection of large volume aliquot (75 µL) of 1-octanol containing the analytes in the reversed-phase chromatography column and MS/MS detection.

The article [9] describes development and validation of a new rapid UPLC-MS/MS method for the quantification of fenspiride in human plasma. The developed method satisfied all regulatory requirements and was successfully applied to the bioequivalence study of coated tablets of fenspiride. Reported methods are sensitive to quantitate the trace level amount of fenspiride hydrochloride present in swab samples. A literature survey revealed that no validated cleaning method for fenspiride hydrochloride is to be found. Due to their high sensitivity and selectivity, analytical methods such as liquid chromatography were previously used for the determination of residues to control cleaning procedures [10].

The aim of this work covered development and validation of the HPLC method for the estimation of trace level residues of fenspiride hydrochloride on pharmaceutical manufacturing equipment surfaces. The developed method was validated with respect to specificity, linearity, limit of detection, accuracy and precision.

Experimental part

Chemicals and reagents

The certified fenspiride hydrochloride, the working standard was supplied by Erregierre S.p.A., Italy. The HPLC grade methanol and analytical grade sodium dihydrogen phosphate monohydrate and orthophosphoric acid were purchased from Merck. High purity water was prepared by using Millipore Direct-Q water purification system (Millipore, USA). Swabs (Alpha® Sampling Swab TX 715) for sampling were purchased from Texwipe company (China).

Apparatus

The chromatography analysis was performed using a Agilent 1260 LC System (Agilent Technologies, USA) equipped with a UV/visible detector. The pH of the solutions were measured by a pH meter (Seven Easy, Mettler-Toledo, Switzerland) with a glass electrode. All measurements were performed at room temperature (22°C).

Chromatographic conditions

The method was validated using an Zorbax Eclipse XDB-C₁₈ (0,15 m x 4,6 mm)

5 μm column with a isocratic mobile phase containing a mixture of 2.76 g/L sodium dihydrogen phosphate monohydrate and methanol (80:20 v/v), pH adjusted to 3.0 with orthophosphoric acid. The mobile phase was filtered through nylon 0.22 μm membrane filters and degassed. The flow rate of the mobile phase was 1.5 ml/min with a column temperature of 30°C and detection wavelength at 210 nm. Time chromatography is a 5 min, injection volume - 50 μl .

Standard solution preparation

A stock solution containing 1.0 mg/mL fenspiride hydrochloride was prepared by an dissolving appropriate amount of drug in water. The final concentration of solution was 10.0 $\mu\text{g/mL}$ of fenspiride hydrochloride. Appropriate dilutions were made with diluent to obtain solution containing 1.0, 3.0, 5.0, 7.0, 10.0, 20.0, 30.0, 50.0, and 100 $\mu\text{g/mL}$.

Sample preparation (extraction procedure)

The selected surfaces (10 \times 10 cm^2) of stainless steel, previously cleaned and dried, were sprayed with 100 μL and 200 μL of standard solution (0.25 mg/mL), for the positive swab control at two concentration levels and the solvent was allowed to evaporate. The total surface were successively wiped first in horizontal and secondly in a vertical way, starting from outside toward the center, with one or two swabs moistened with water to remove the residue from the surface. The swabs

were placed in the 25 mL screw-cap test tubes containing 5 mL water (5 $\mu\text{g/mL}$ and 10 $\mu\text{g/mL}$). The tubes were placed in an ultrasonic bath for 15 min, and the solutions were analysed by HPLC.

Results and discussion

Acceptance limit calculation

The acceptable limit for the drug residue must ensure the absence of cross contamination for subsequent batches manufactured in the affected equipment.

The maximum allowable carryover (MACO) is the acceptable transferred amount from the previous to the following product. The MACO is determined based on the therapeutic dose, toxicity and generally 10 ppm criterion. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the residue limit in terms of the contamination level of active ingredient per surface area of equipment. The total surface area of the equipment in direct contact with the product was accounted for in the calculation. The limit per surface area was calculated from the equipment surface area and the most stringent maximum allowable carryover. The 0.1% dose limit criterion is justified by the principle that an active pharmaceutical ingredient (API) at a concentration of 1/1000 of its lowest therapeutic dose will not produce any adverse effects on human health [11].

The calculated limit per surface area in the case

of FH was 40.0 µg/swab pro 100 cm². A stainless steel surface area of 10 cm×10 cm was chosen for practical reasons.

Method development and optimization

The main objective in this study has been to develop an HPLC method using isocratic conditions for the analysis of low quantities of fenspiride hydrochloride, trying to get a high peak in a short time. We selected 210 nm for the analysis because the drug has sufficient absorption and low quantities of FH may be detected correctly. Furthermore, the calibration curves obtained at 210 nm show good linearity. The mobile phase very often used is the mixture of phosphate buffer and methanol in different proportions. Several mobile phases were tested, varying their composition and pH, to obtain the chromatographic separation. The sufficient tailing factor and plate number were achieved with the proposed mobile phase composed by solution 2.76 g/L sodium dihydrogen phosphate monohydrate and methanol (80:20 v/v), pH adjusted to 3.0 with orthophosphoric acid gave best resolution and sensitivity with a very shorter run time (5 min). An Zorbax Eclipse XDB-C₁₈ (0,15 m x 4,6 mm) 5 µm column was selected over an Zorbax Eclipse XDB-C₁₈ (0,25 m x 4,6 mm) 5 µm column, to achieve good peak shape and symmetry. The injection volume was varied between 10 and 100 µL, finally 50 µL was chosen, because bigger volumes imply wider

peaks without much enhancement of the signal-to-noise ratio. The flow rate of the mobile phase was kept 1.5 mL/min and the column temperature was maintained at 30 °C.

Validation of the method

In accordance with the ICH-Guidelines on the validation of analytical methods [12] the following validation characteristics were examined: specificity, linearity, limit of detection, and limit of quantification, precision, and accuracy.

System suitability

The system suitability test was used to ensure that the HPLC system and procedures are adequate for the analysis performed. Parameters of this test were column efficiency (number of theoretical plates), asymmetry of chromatographic peak, and reproducibility as RSD of peak area of six injections of standard solution (10.0 µg/ml). During performing the system suitability test, in all cases relative standard deviation (RSD) of the peak areas was ≤2.0%, the number of theoretical plates per column was 4000, and the USP tailing factor was ≤2.0.

Specificity

The ability of this method to separate and accurately measure the peak of interest indicates the specificity of the method. The specificity of the method was checked by injecting fenspiride hydrochloride standard, fenspiride hydrochloride sample, the background control sample, and the negative swab control. There is

no interference from the extracted blank swab, the extraction solvent and excipients used for the drug product (tablets) at the retention time of analyte peak (**Figure 1**).

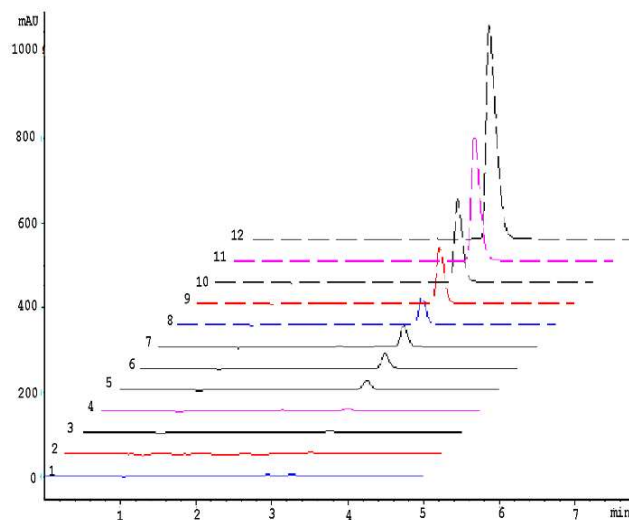


Figure 1. Overlay chromatograms of extracted blank swabs (1), extraction solvent (2), excipients used for the drug product (3), and the active compound at a concentration range from 1.0 to 100.0 $\mu\text{g/ml}$ (4-12).

Linearity

The linearity of the method was established by injection of standard solutions at nine concentration levels (**Figure 1**) over a wide concentration range from 1.0 to 100.0 $\mu\text{g/mL}$. The calibration curve (**Figure 2**) was constructed by plotting the response area against the corresponding concentration injected, using the least square method. The calibration curve values of slope, intercept, and correlation coefficient for fenspiride hydrochloride are 46.09218, 4.78055 and 0.99994, respectively. The high value of the correlation coefficient indicated good linearity.

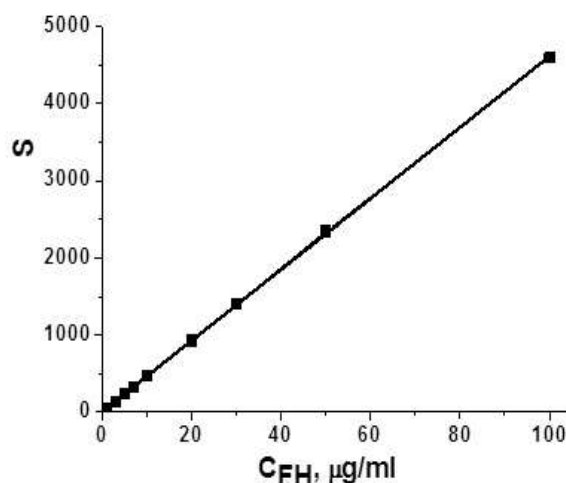


Figure 2. Calibration curve for determination of FH.

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were determined based on the standard deviation of the response (y-intercept) and the slope of the calibration curve at low concentration levels according to ICH guidelines. The LOD and LOQ for fenspiride hydrochloride were found to be 0.41 $\mu\text{g/ml}$ and 1.25 $\mu\text{g/ml}$, respectively.

Precision and accuracy

The precision and accuracy of the proposed cleaning validation procedure, reported as relative standard deviation (RSD) and the recovery (%), respectively, were assessed by comparing the amount of analyte determined versus the known amount spiked at two different concentration levels (5.0 and 10.0 $\mu\text{g/mL}$) with 3 replicate ($n=3$) for each investigated concentration level. The recovery and the RSD values (**Table 1**) for each level

illustrated good precision and accuracy of the method. These precision and recovery results are excellent for the purpose of residue monitoring.

Table 1. Results of the recovery study

Sample	Spiked level (µg/mL)	Recovery, % (n=3)	RSD, %
stainless steel plate	5.0	90.04	3.89
	10.0	91.82	3.17
swabs	5.0	97.24	1.54
	10.0	98.15	1.68

Solution stability

The stability of the fenspiride hydrochloride in the swab matrix and standard solution (10.0 µg/mL) was tested. The spiked sample and standard solution were stored at room temperature for 2 days. All the samples were injected into the HPLC system after 24 and 48 h against freshly prepared standard solution. Sample and standard solution were stable up to two days. No changes in the chromatography of the stored samples were found, and no additional peaks were registered when compared with the chromatograms of the freshly prepared standard solution.

Assay of swab samples

Swab samples from different locations of tablet press have been analyzed to determine the residual of FH. These samples were prepared and analyzed by the proposed method. Some of the results obtained for these samples are presented in **table 2**.

Table 2. Determination of FH in actual swab samples collected from different locations of tablet press (100 cm²)

Sample no	MACO, µg	Results, µg
1	40	7.45
2		Not detected
3		10.23
4		24.17
5		9.56

The results of determination of trace amounts of FH on the tablet press surface evidence of satisfactory quality cleaning of the equipment.

Conclusions

A new sensitive HPLC method has been developed for the determination of fenspiride hydrochloride residues on the pharmaceutical manufacturing surface to control the efficiency of the equipment cleaning. The suggested method was validated in accordance with ICH guidelines and has the advantage of being simple, sensitive and suitable for routine analysis in a quality control laboratory. Stability studies show that FH samples were, at least stable over the investigated 48 hours.

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