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Development and validation of an LC assay for sumatriptan succinate residues on surfaces in the manufacture of pharmaceuticals

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Abstract

A high performance liquid chromatographic (HPLC) method for the assay of sumatriptan succinate residues in swabs collected from manufacturing equipment surfaces was developed and validated in order to control a cleaning procedure. The swabbing procedure using two cotton swabs moistened with water was validated applying a wipe-test and a HPLC method developed to determine low quantities of the drug. The HPLC method involves a C18 column at 25 °C, a mixture of ammonium phosphate monobasic (0.05 M)–acetonitrile (84:16, v/v) as a mobile phase and UV detection at 228 nm. Using the proposed method, the average recoveries obtained are of 88.5% for vinyl, 94.2% for glass and 95.2% for stainless steel plates with RSD of 5.5 ($n = 36$), 2.3 ($n = 36$), 2.2% ($n = 36$), respectively. The method was successfully applied to the assay of real swab samples collected from the equipment surfaces.

Keywords: Sumatriptan succinate; Swab analysis; Cleaning validation; HPLC

1. Introduction

Sumatriptan succinate is chemically designated as 3-[2-(dimethylamino)ethyl]–*N*-methyl indole-5-methanesulfonamide succinate (1:1). The structure of the active pharmaceutical ingredient is shown in Fig. 1. Sumatriptan succinate is a white to off-white powder that is readily soluble in water and in saline. Sumatriptan is an agonist for a vascular 5-hydroxytryptamine receptor subtype. Its activity in humans is included in the treatment of migraine headache.

An important step in the manufacture of pharmaceutical products is the cleaning of equipment and surfaces. The cleaning procedures for the equipment must be validated according to good

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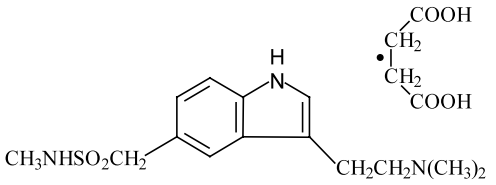

Fig. 1. Structure of sumatriptan succinate.

manufacture practice (GMP) rules and guidelines [1]. The main objective of cleaning validation is to avoid contamination between different productions or *cross contamination*, the verification of this cleaning is carried out by determining the amount of residues in surfaces involved in the manufacture process. There are several acceptance criteria based on logical and scientific rationale, they mainly depend on the drug type and the dosage way. The limits will be lower in the case of more active drugs [2,3].

Nowadays, residue determination in equipment and production areas is being a very important aim and it is considered the first step before beginning the production of the next batch. The recovery of the drug is carried out using a cotton swab moistened with different solvents. This method depends on various parameters like the surface type (glass, vinyl, stainless steel …) [4–8], and it is necessary to establish the way of addition of the drug on the different surfaces and the procedure to collect it [9,10].

To determine sumatriptan succinate, there are several analytical methods proposed, including HPLC with UV/Vis [11–16], MS [17–21] and electrochemical detection [22,23]. The most recommended are methods that include HPLC on C18 columns [11,16,17,19,20,22,23], with different mobile phases and detection in UV region. The drug determination is also carried out by capillary electrophoresis [24,25].

Taking this information into account, we have developed and validated a simple method that allow us to evaluate the possible residual drug after removing it from surfaces of pharmaceutical manufacture areas (glass, vinyl and stainless steel), using our previous experience in the study of the acetylsalicilic acid [26] and ranitidine hydrochloride [27].

2. Experimental

2.1. Chemicals

The sumatriptan succinate certified standard (98.6% w/w) and the plates of different materials were generously given by Glaxo Wellcome Factory in Aranda de Duero (Spain). Acetonitrile HPLC–UV grade was obtained from Lab-Scan (Dublin, Ireland). Ammonium phosphate monobasic (98–102%, w/w), glacial acetic acid, sodium hydroxide, ortophosphoric acid (85%, v/v), all of analytical grade, were purchased from Scharlau (Barcelona, Spain). 0.45 μm Nylon filters were obtained from Millipore (Bedford, MA, USA). Ultrapure water was obtained in a Milli-RO plus system together with a Milli-Q system from Millipore (Bedford, MA, USA). Absorbent cotton 100% from LauSan (Valladolid, Spain).

2.2. Equipment

The HPLC system used in this study consisted of a vacuum degasser, a quaternary pump, an automatic injector with a column oven and a photodiode array detector, all HP Model 1100, from Agilent Technologies (Palo Alto, CA, USA) controlled by an HP Chemstation software. An AE-240 analytical balance from Mettler (Toledo, USA), and a Bransonic five ultrasonic bath from Scharlau (Barcelona, Spain).

2.3. Chromatographic conditions

The mobile phase was a mixture of ammonium phosphate monobasic (0.05 M)–acetonitrile (84:16, v/v), pH* 3.3. The flow rate was set at 1.0 ml/min. Column temperature was 25 °C. The injection volume was 50 μl and the detection wavelength was set at 228 nm.

The chromatographic separation was carried out on a 15 cm × 4.6 mm ID, 4 μm C18 LUNA column obtained from Phenomenex (Torrance, CA, USA).

Table 1
Quantitative calibration, sensitivity, repeatability, reproducibility and accuracy values obtained for the quantitative determination of sumatriptan succinate

Parameters	
Range (μg/ml)	0.009–14
Intercept (b)	−0.05
S_b	3.14
Slope (a)	323.64
S_a	0.55
LOD	3 ng/ml (RDS = 3.8)
LOQ	9 ng/ml (RDS = 2.8)
Repeatability (RSD%, $n = 5$)	0.05
Reproducibility (RDS%, $n = 15$)	1.4
Accuracy (error%, $n = 15$)	0.5
Purity peak (factor)	990–1000

2.4. Preparation of calibration standards

A sumatriptan succinate stock solution was prepared by accurately weighing sumatriptan succinate reference standard (5 mg approximately) and transferring it into a 50 ml volumetric flask. This standard was dissolved in acetic acid (0.1 M), dilutions were later prepared with the same solvent to obtain the solutions for calibration.

2.5. Sample preparation

Two cotton swabs of approximately 0.25 g were taken and rinsed exhaustively with water and acetonitrile and then dried under vacuum. After that both dried cotton swabs were placed into a 50 ml screw cap plastic test tube and weighed.

The selected surface (20×20 cm^2; 10×10 cm^2) of glass, vinyl and stainless steel, previously cleaned and dried, was sprayed with 1 ml of standard solution of sumatriptan succinate, and the solvent (acetic acid 0.1 M) was allowed to evaporate. The surface was wiped with the first cotton swab (taken from the tube) soaked with water, passing it in various ways (horizontally, vertically, back and forwards), and the other dry cotton swab was used to wipe the wet surface. The two swabs were placed in the tube and acetic acid was added to reach a mass 7 g higher than the one obtained before. After that, the tube was placed in the ultrasonic bath for 10 min and the solution was analyzed by HPLC.

3. Results and discussion

3.1. Development of the chromatographic method

The main objective in the present study has been to develop an HPLC assay using isocratic conditions for the analysis of low quantities of sumatriptan succinate, trying to get a high peak in a short time, because it is not expected to find other compounds retained on the surfaces.

We select 228 nm for the analysis because the drug has sufficient absorption and low quantities of sumatriptan succinate may be detected correctly. Furthermore, the calibration curves obtained at 228 nm show good linearity.

A mobile phase very often used is the mixture of methanol–ammonium acetate in different proportions. This mobile phase did not seem to be adequate for the determination of trace levels of sumatriptan succinate because of the big front that originates. To solve this problem, several mobile phases were tested, varying their composition and pH, to obtain the chromatographic separation. The proposed mobile phase composed by ammonium phosphate monobasic (0.05 M)–acetonitrile (84:16, v/v), adjusted to pH* 3.3 with o-phosphoric acid (1.0 M), gave the best resolution and sensitivity. Under the described conditions the sumatriptan succinate peaks were well defined, resolved and free from tailing.

The injection volume was varied between 10 and 100 μl, finally 50 μl was chosen, because bigger volumes implied wider peaks without an enhancement of the signal to noise ratio. The temperature was also modified between 25 and 60 °C, the increase in temperature did not imply an enhancement in chromatographic parameters, as a result, a temperature of 25 °C was selected.

3.2. Validation of the chromatographic method

The validation of chromatographic method was carried out by determining the selectivity, linearity, precision, accuracy, quantitation and detection limits and stability of the analytical solutions. Results are listed in Tables 1 and 2.

Fig. 2 shows the chromatograms obtained from a blank swab and a swab sample. No interference was observed with the analysis of swab samples. The selectivity has been demonstrated considering the purity peak information.

The detection limit (LOD) and quantitation limit (LOQ) were determined by measuring the magnitude of the analytical background response, by injecting a number of blank swab samples ($n = 5$), calculating the mean (0.027 mUA) and RSD = 15%. The response standard, plus three times the mean background response provided the LOD (see Table 1). The chromatograms obtained are shown in Fig. 3.

The precision (repeatability and reproducibility) and accuracy of the method were determined by injecting the same standard of sumatriptan succinate five times (precision), and five different standards (accuracy). The analytical result was compared with the known added value.

The stability of the standard solutions was determined by preparing a set of standard solutions,

Table 2
Stability results obtained for sumatriptan succinate in standards (a) and swab samples (b)

	Recovery (%) ± RSD (%) ($n = 5$)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 8
(a)							
Light-20 °C		100.3 ± 0.03	99.9 ± 0.05	99.6 ± 0.05	99.6 ± 0.1	98.6 ± 0.07	98.8 ± 0.04
Dark-20 °C	100 ± 0.06	100.4 ± 0.04	100.1 ± 0.07	100.5 ± 0.08	99.3 ± 0.2	98.4 ± 0.04	99.5 ± 0.02
Dark-4 °C		100.7 ± 0.03	100.6 ± 0.04	100.7 ± 0.10	100.5 ± 0.1	100.3 ± 0.08	99.8 ± 0.04
(b)							
Light-20 °C[b]		98.4 ± 0.1	95.6 ± 0.1	94.7 ± 0.1	92.5 ± 0.2	90.5 ± 0.1	84.4 ± 0.2
Dark-20 °C	100 ± 0.01	100.7 ± 0.1	100.9 ± 0.1	99.7 ± 0.06	100.0 ± 0.08	99.3 ± 0.1	98.8 ± 0.1
Dark-4 °C		100.1 ± 0.06	100.4 ± 0.08	100.3 ± 0.04	100.2 ± 0.2	100.6 ± 0.2	100.5 ± 0.2

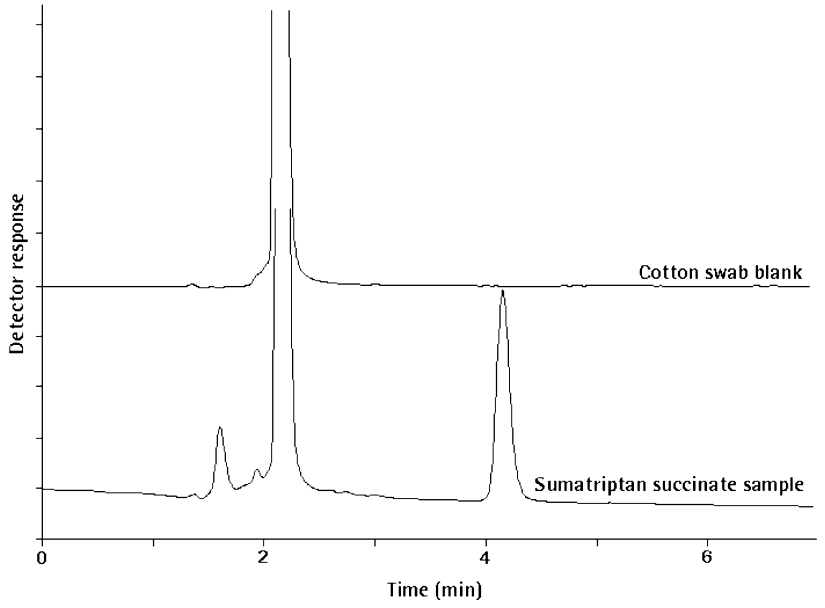

Fig. 2. Chromatograms from a cotton swab spiked with sumatriptan succinate and from a blank.

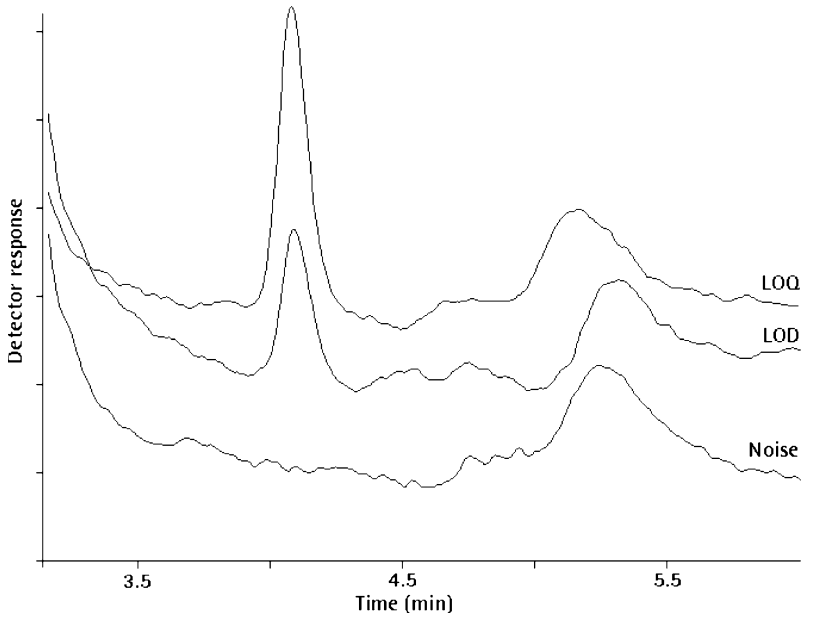

Fig. 3. Chromatograms for limit of detection (3 ng/ml), limit of quantification (9 ng/ml) and blank of acetic acid 0.1 M.

that were stored in three different conditions (dark-20 °C, light-20 °C, dark-4 °C). After 1, 2, 3, 4, 5, 6, 7 days, these aged solutions were reanalyzed against freshly prepared standard solutions. The results demonstrated that actives were stable for at least 7 days.

The stability sumatriptan succinate in the swab samples was determined by spiking swabs directly with solutions of the drug, assaying immediately after their preparation and repeating the same procedure day after day, against freshly prepared standard solutions. After 7 days, the average concentration of samples stored at light 20 °C reduced to 84.4% of its initial concentration. Therefore, it is recommended storing the sample solutions in darkness (see Table 2).

3.3. Sample treatment optimization

Two cotton swabs were rinsed firstly with water and, afterwards, with acetonitrile HPLC grade, then, vacuum dried and, after that, they were sunk in acetic acid 0.1 M and sonicated for 10 min. The extract was analyzed by HPLC and no interference that could disturb the sumatriptan succinate peak was found.

Two cotton swabs were spiked with different quantities of sumatriptan succinate ranging from 1 to 20 μg, and were placed in the tube. In the extraction procedure, four masses (3, 5, 7 and 10 g) of the acetic acid 0.1 M were assayed for each quantity of sumatriptan succinate. The recoveries obtained were 76.1, 96.6, 101.7, and 102.6, respectively, with RSD varying between 19.4 and 3.3%, because of that we select 7 g of acetic acid in order to obtain the best detection and quantitation limits and lowest RSD.

3.4. Sumatriptan succinate recovery from vinyl, glass and stainless steel surfaces

Each plate, previously cleaned with water, was spiked with 1.0 ml of different standard solutions of sumatriptan succinate to obtain 0.3, 1, 10 and 20 μg. The plates were left to dry, and the drug residues were removed by wiping the surface with the cotton swab in a way that assures that the entire plate was thoroughly cleaned.

The effect of the surface size on the recovery was studied. The results obtained for plates of 10×10 and 20×20 cm^2 showed that the recover-

ies were higher and the RSD were lower when using 10×10 cm^2 surfaces. Results are listed in Table 3.

Applying an ANOVA test of two tails ($P = 0.95$) to the results obtained using the two cotton swabs, it was found that the recovery was influenced by the type and the size of surface, and not by the level of the drug spiked, getting the lowest recoveries from the vinyl surface.

Table 3
Recovery of sumatriptan succinate added from stainless steel, glass and vinyl surfaces (100 and 400 cm^2)

100 cm^2	R (%) ± RSD (%)		
	Stainless steel	Glass	Vinyl
μg added ($n = 9$)			
0.3	94.6 ± 2.1	96.3 ± 2.5	90.3 ± 3.4
1	94.7 ± 1.8	91.5 ± 2.6	92.3 ± 3.8
10	95.1 ± 1.8	94.4 ± 0.6	82.0 ± 4.4
20	95.6 ± 2.2	94.3 ± 1.2	89.2 ± 3.5
($n = 36$)	95.2 ± 2.2	94.2 ± 2.3	88.5 ± 5.5
400 cm^2 μg added ($n = 5$)			
1	91.7 ± 2.8	87.3 ± 5.3	73.6 ± 4.1
10	87.0 ± 3.6	88.4 ± 3.0	73.5 ± 8.0
20	91.9 ± 7.7	89.2 ± 6.3	71.8 ± 7.0
($n = 15$)	90.2 ± 5.0	88.3 ± 4.7	73.0 ± 6.2

Table 4
Recovery of sumatriptan succinate, from 10×10 cm^2 surfaces in relation to the different solvents added to the first cotton swab

Solvent ($n = 15$)	Recovery (%) ± RSD (%)		
	Stainless steel	Glass	Vinyl
Water	95.2 ± 1.9	93.2 ± 2.3	87.8 ± 5.5
Water 60 °C	95.8 ± 2.5	92.0 ± 2.7	85.1 ± 5.4
CH_3COOH 0.5% v/v	93.1 ± 1.5	89.3 ± 4.0	84.6 ± 4.8
NaOH (pH 10)	61.5 ± 5.9	62.6 ± 4.9	61.2 ± 3.5
CH_3OH	69.6 ± 9.1	62.4 ± 6.4	47.5 ± 7.0
No solvent	73.1 ± 14.3	63.6 ± 8.5	4.8 ± 2.8

To study the influence of the solvent used with the first cotton swab on the analyte recovery, the surfaces were spiked with different quantities of sumatriptan succinate, and different types of solutions (water, water 60 °C, acetic acid 0.1 M, NaOH 10^{-4} M, methanol) were used to wet the cotton swab. Blank ones were also tested. As it can be observed in Table 4, recoveries ranged from 5% in the vinyl surface using two dry cotton swabs, to 95.8% in stainless steel surfaces using two cotton swabs, the first one wetted with water at 60 °C.

The effect of temperature and pH of the solvent for the first cotton swab on the drug recovery were also studied, the highest recoveries were obtained with acidic values of pH and no differences were found on recoveries using water at 25 or 60 °C. It could be appreciated that in all cases, the highest recoveries were obtained for stainless steel plates and the lowest for vinyl plates.

Analyst-dependent (intermediate) precision was determined by a repeat assay of accuracy/recovery experiments by three different analysts. The recovery results for stainless steel ($n = 5$) were 92.1, 94.9 and 95.1%, that should the good reproducibility of the procedure.

4. Conclusions

A sensitive high performance liquid chromatographic cleaning validation method for the determination of residual levels of sumatriptan succinate in swab samples collected by wiping different surfaces within the manufacture area has been developed and found to be accurate and precise. The detection limit is 3 ng/ml and the calibration curve is linear over the concentration range of 0.009–14 μg/ml. No interference was observed while injecting the diluent or the swab blank extracts, the stability is longer than 8 days when samples and standards are stored in darkness at 4 °C. The highest recoveries are obtained on stainless steel plates and the lowest on vinyl. Those recoveries are strongly dependent on the surface sampled and also, on the solvent used to wet the cotton surface.

Repiclated recovery studies on a different system by two other analysts demonstrated the precision of the method, and proved that more than 87% of the active substance was recovered on the stainless steel surfaces.

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