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Validation of the removal of acetylsalicylic acid Recovery and determination of residues on various surfaces by high performance liquid chromatographic

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Abstract

The validation of a procedure to clean glass, vinyl and stainless steel surfaces that have been exposed to acetylsalicylic acid during its manufacture is described. The cleaning procedure using two cotton swabs moistened with the mobile phase was validated using a wipe-test and a high-performance liquid chromatography (HPLC) method developed to determine low quantities of the acid. The HPLC method involves an octadecylsilane column at 55°C, a mixture of water–acetonitrile–orthophosphoric acid (779:220:1, v/v) as mobile phase and detection at 226 nm. Recoveries of 86%, 90% and 94% were obtained from vinyl, glass and stainless steel plates respectively. The validation gave acceptable levels of sensitivity, recovery, precision and linearity.

Keywords: Validation; Pharmaceutical analysis; Acetylsalicylic acid

1. Introduction

In pharmaceutical manufacturing it is well established that equipment and production areas must be thoroughly cleaned after each manufacturing campaign, regulatory authorities recommend that those procedures must be validated [1–6]. Cleaning validation is the process of assuring that cleaning procedures effectively remove the residues from manufacturing equipment/facilities below a predetermined level, usually related to the dose. This is necessary not only to assure the quality of future products but also to prevent cross-contamination and also as a World Health Organization Good Manufacturing Practice requirement. Among the most important components of any cleaning validation strategy the methods used to wipe the surfaces and to measure residuals have an special importance.

Acetylsalicylic acid (ASA) is a very common compound, moderately water soluble, that appears as main ingredient in many formulations, being the aspirin the most well known, and it is manufactured world wide by many drug companies. To determine ASA, apart from acid-base titration, usually HPLC methods are recommended, generally using C_{18} columns with acidic mobile phases at room temperature and detection at 275 nm, although obviously there are other alternatives [7–12]. The sensitivity of those methods is not frequently appropriate when measuring low quantities of ASA is needed because those methods are not focused to determine trace levels, as happens in cleaning validation studies, so it is necessary to have methods adapted to those concentrations.

For sampling the residues from the surfaces,

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several methods have been proposed although according to the US Food and Drug Administration (FDA) guide the most desirable is the direct method [13,14], using a swabbing technique that involves the employ of a swabbing material, often saturated with a solvent. For water soluble components it seems to be more advisable to choose pre-washed cotton as material [15], that can be used in different ways, being the double swab technique preferred because it usually gives bigger recoveries [16].

In this study procedures are described for validating the wipe-testing of vinyl, glass and stainless steel surfaces and for quantitatively determining the ASA residue present. Those procedures will ensure that equipment/facilities used have been cleaned to acceptable levels.

2. Experimental

2.1. Chemicals

Methanol and acetonitrile both of HPLC UV-grade were obtained from Lab-Scan (Dublin, Ireland). Orthophosphoric acid (85%) and glacial acetic acid of analytical grade were purchased from Scharlau (Barcelona, Spain). Acetylsalicylic acid certified standard and purified wheat starch were obtained from Sigma–Aldrich Química (Madrid, Spain). Ultrapure water was obtained in a Milli-RO plus together with a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Equipment

The HPLC system consisted of an SCM vacuum degasser, a P4100 quaternary pump, an AS3100 automatic injector with column oven, an SM5000 photodiode array detector and a Lctalk software (ver.2.03.01) all obtained from Thermo Separation Products (San José, CA, USA). A Bransonic 5 ultrasonic bath was purchased from Scharlau (Barcelona, Spain). An AE-240 analytical balance was obtained from Mettler (Toledo, OH, USA).

2.3. Column

Chromatographic separation was carried out on a LiChrospher 100 RP-18, 5 μm, 125×4 mm column obtained from Merck (Darmstadt, Germany).

2.4. Chromatographic conditions

The mobile phase was a mixture of water–acetonitrile–orthophosphoric acid (790:220:1, v/v). The flow-rate was 1 ml/min and the oven temperature 55°C, the injection volume was 25 μl and the detector was set at 226 nm.

2.5. Standard solutions

A stock solution of acetylsalicylic acid was prepared by dissolving 5 mg of ASA accurately weighed in 50 ml of a mixture methanol–glacial acetic acid (95:5, v/v). this solution was further diluted in the mobile phase to obtain the appropriate concentrations for calibration.

2.6. Sample preparation

Take cotton swabs of approximately 0.25 g. Rinse exhaustively with the mobile phase and then left dry under vacuum. Place two dried swabs into a 50 ml screw cap plastic test tube and weigh.

Spike the selected surface (20×20 cm^2) by spraying 1 ml of the ASA standard solution. Leave the plate to be dried.

Take a dried cotton swab (from the tube) and drop it into the mobile phase (250 ml erlenmeyer flask), with a stainless steel extra-long forceps press to remove the excess of mobile phase.

Wipe the surface firstly with the wet swab, passing it in different ways, after that repeat the operation with the dried swab.

Place again the two swabs into the tube and add mobile phase to reach a weight 5 g higher than the one obtained before.

Press with the aid of forceps the swabs to leach the collected ASA, then place the tube in the ultrasonic bath for 5 min.

Take the solution and inject into the HPLC system.

3. Results and discussion

3.1. Optimization of chromatographic conditions

To obtain the best overall chromatographic conditions the mobile phase, the wavelength and the temperature were optimized.

The mobile phase that is usually employed to analyse ASA in pharmaceutical formulations at relative high concentration is a mixture of water–methanol–acetic acid (69:28:3, v/v), where the acetic acid contributes to reduce the tailing of the ASA peak, that is detected at 275 nm, working at 27°C. With those conditions it is not possible to measure low ASA concentrations being necessary to modify the method. In Table 1 the results of the experiments are summarized.

In the ASA spectrum there is a higher peak at 226 nm with great molar absorptivity, so for trace level analysis this wavelength will be more favourable. Changing to this wavelength it could be observed that the chromatographic signal was enhanced and the background as well. As the acetic acid absorbs also at 226 nm, experiments were made reducing their proportion in the mobile phase, that led to wider peaks and longer retention times, when the acetic acid was eliminated from the mobile phase the ASA peak practically disappeared. See Table 1.

As the orthophosphoric acid had been also used in mobile phases for determining high ASA concentrations, we test this acid instead of the acetic acid. With an 0.1% of H_3PO_4 in the mobile phase (pH 2.2) the ASA peak was higher and narrower, showing a stable baseline.

Changing the methanol by acetonitrile in the same proportion the peak profile was also enhanced, the retention times were shortened (1.71 min). Taking into account the analysis of real samples we preferred to reduce the acetonitrile percentage till 22% (5.6 min).

The oven temperature was also varied from 27°C to 65°C, this increasing in the temperature implied a big increase in the area and height of the ASA peak, reducing gradually the retention time. A temperature of 55°C was selected.

In Fig. 1 two chromatograms obtained with the initial and the selected conditions are shown. As it can be seen not only the ASA peak is more adequate but also the peaks belonging to the front have been diminished. The front that appears in Fig. 1b is originated by the acetic acid used to prepare the standard solution, acetic acid is present because ASA standard is not dissolved in acetonitrile or in the mobile phase whereas it is dissolved very well in the methanol–acetic acid mixture, so the solution adopted was: to prepare stock solutions in methanol–acetic acid and make dilutions with the mobile phase, so the negative effect of the acetic acid will be reduced.

3.2. Validation of the chromatographic method

The chromatogram obtained for a standard of acetylsalicylic acid is shown in Fig. 1b. The instrument precision, determined by eighty successive injections of the standard preparation, exhibited a maximum RSD (t_R) of 0.18%. The column efficiency was greater than 2800 theoretical plates. The tailing factor was not more than 1.2 at 5% peak height.

3.3. Linearity

An acceptable correlation coefficient of 0.99996 was obtained for the detector response plotted against drug concentration ranging between 0.15 and 10 mg/l. Eighty standard solutions were prepared

Table 1
Effects of changing proportions and compositions of the mobile phase on the chromatographic peak of an ASA standard 27°C and 275 nm

	Water–MeOH–AcH				Water–MeOH–H_3PO_4	Water–acetonitrile–H_3PO_4			
	69:28:3	70:28:2	71:28:1	72:28:0	(71.9:28:0.1)	71.9:28:0.1	75.9:24:0.1	77.9:22:0.1	77.9:20:0.1
Area	11 202	9340	8215	6913	11 883	14270	13851	13581	12288
Width (5%)	0.56	0.71	0.85	0.93	0.43	0.27	0.29	0.31	0.37
t_R (min)	6.31	6.56	7.21	8.02	6.02	1.71	4.61	5.28	6.73

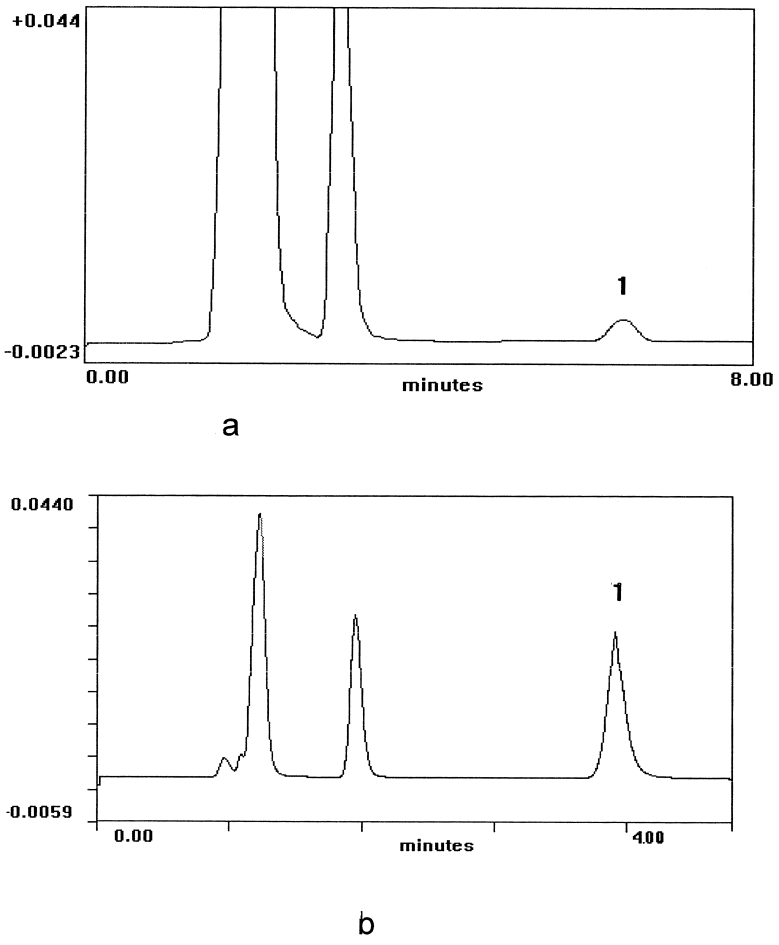

Fig. 1. (a) Chromatogram obtained from a 25 μg/ml ASA standard with the initial conditions: 275 nm, 27°C and water–MeOH–AcH (69:28:3, v/v); (1) acetylsalicylic acid. (b) Chromatogram obtained from a 5 μg/ml ASA standard with the selected conditions: 226 nm, 55°C and water–ACN–H_3PO_4 (790:220:1, v/v); (1) acetylsalicylic acid.

and each solution was injected by duplicate. Typical regression parameters were: *a* (slope), *b* (*y*-intercept), $a=41649$ $s_a=73.3$, $b=-674$ and $s_b=355$ respectively and $s_{y/x}=1.25\ e^{03}$.

3.4. Selectivity

Any interference was not found in the application of the method. To prove that, some swabs were contaminated with wheat starch, frequently present in formulations with ASA, and then submitted to the procedure. In Fig. 2 the chromatogram obtained is shown and it can be observed that there is no interference.

3.5. Precision and accuracy

The precision of the HPLC method was evaluated by measuring the peak chromatographic area of acetylsalicylic acid, 10 times on the same standard (μg/ml). The coefficient of variation was 1.16%. Injection precision less than 5% RSD are considered appropriate for these trace level determinations.

The accuracy of the method was estimated by injecting five different standards of the same con-

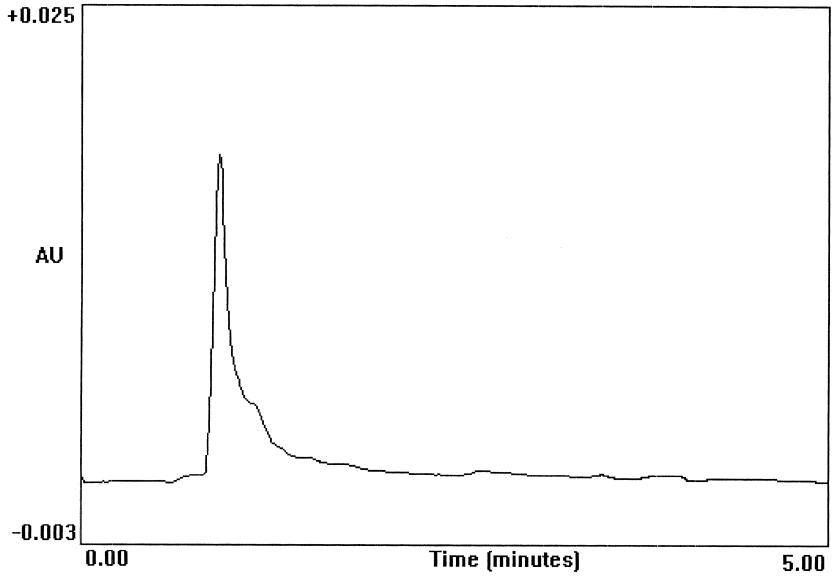

Fig. 2. Chromatogram obtained from a non spiked cotton swab contaminated with wheat starch.

centration (1 μg/ml) obtaining a recovery of 99.6% with an RSD of 1.6%.

3.6. Limits of detection and quantification

Limits of detection and quantification were calculated on the basis of chromatograms for a blank and an acetylsalicylic acid calibration solution, see Fig. 3. A limit of detection of 0.04 μg/ml and a limit of quantification 0.14 μg/ml were obtained.

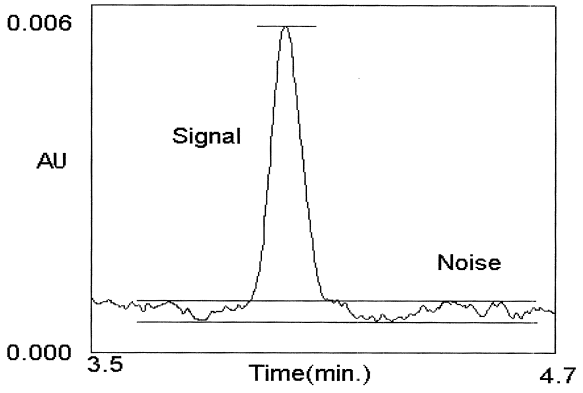

Fig. 3. Limit of quantification. Chromatogram for a 0.14 μg/ml ASA standard.

3.7. Extraction procedure optimization

Cotton swabs of 0.25 g were firstly rinsed with HPLC methanol grade and then vacuum dried after that, they were sunk in the mobile phase and sonicated for 10 min. The dissolution was analysed by HPLC and no peak appeared that could disturb the possible ASA peak.

Recovery of ASA from previously cleaned cotton swabs was established by spiking the swab with several quantities of ASA (5–50 μg). Placing the swab in a tube, weighing and adding mobile phase till reach a final mass of 3 g, 5 g and 8 g higher; after pressing the swab and sonication, the dissolution was analysed obtaining the results shown in Table 2. As it can be seen it seems to be advisable the use of 5 g of mobile phase (c.a. 5.4 ml taking into account its density), because lower quantities do not wet completely the swab and higher volumes implied a non necessary dilution of the sample.

3.8. Analytical performance

Table 3 shows the results obtained in the analysis of the extracts from cotton swabs spiked with

Table 2
Recovery of ASA from cotton swabs

Mobile phase added (g)	Drug added ($n=5$)					
	5 (μg)		25 (μg)		50 (μg)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
3	92.8	7.5	92.6	6.8	93.9	8.3
5	99.4	3.8	98.1	1.8	97.4	2.1
8	98.1	4.1	99.5	3.7	98.2	2.0

different quantities of ASA (0.78 μg, 10.2 μg and 26.7 μg), the average recovery was 98.5%.

The RSD values ranged from 1.6% for a quantity of 10.2 μg (intra-day) to 5.6% for 0.78 μg in the inter-day study.

3.9. ASA recovery from vinyl, glass and stainless steel surfaces

After cotton swab recovery had been established, recovery from vinyl, glass and stainless steel surfaces was determined. Each 20×20 cm^2 pre-cleaned plate (plates without spiking were submitted to the overall procedure and the final dissolution was considered as the blank) was spiked by spraying 1.0 ml of different ASA solutions (1 μg/ml, 10 μg/ml and 20 μg/ml, in methanol–acetic acid). 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 (horizontally, vertically and diagonally, back and forth).

A dry cotton swab, a cotton swab moistened in the mobile phase and two cotton swabs (the first one was moistened in the mobile phase and the second was dry) were tested. In Table 4, the results obtained are shown. As it can be appreciated the best procedure is the double swab technique because higher recoveries are always obtained. The % recovery of total drug was equivalent in the three concentrations investigated. In relation to the surface material it can be also appreciated that the highest recoveries are obtained for stainless steel plates. In the case of vinyl it is interesting to emphasize that recoveries gradually diminish when the plate has been used in different tests for a long time (more than 6 months), because of that and to compare results with other surfaces it is very convenient to carry out the study on new plates.

4. Conclusions

An isocratic high-performance liquid chromatographic method to determine acetylsalicylic acid residuals in manufacturing pharmaceutical production surfaces has been developed. The validation of the method demonstrated acceptable levels of sensitivity, precision, linearity and selectivity.

To extract the acetylsalicylic acid from surfaces a wipe test using two cotton swabs is recommended. The recoveries obtained from vinyl, glass and stainless steel surfaces are near 90% or higher and there is no interference from the cotton swab.

Table 3
Intra- and inter-day variation of the method for ASA

Conc. added (μg)	Intra-day ($n=10$)			Inter-day ($n=10$)		
	Conc. found (μg)	Error (%)	RSD (%)	Conc. found (μg)	Error (%)	RSD (%)
0.78	0.76±0.03	2.6	4.8	0.73±0.04	6.4	5.6
10.2	10.1±0.12	0.9	1.6	9.91±0.29	2.9	3.1
26.7	26.4±0.25	1.1	1.9	26.1±0.53	2.3	4.2

Table 4
Recovery of ASA from vinyl, glass and stainless steel plates

Sampling	Drug added	Vinyl		Glass		Stainless steel	
	(μg) $n=5$	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Dry cotton swab	1	[a]	[a]	22.7	15.1	19.9	10.5
	10	[a]	[a]	18.5	23.2	22.4	19.8
	20	[a]	[a]	20.3	18.7	27.6	23.2
Wet cotton swab	1	73.5	14.2	67.7	16.2	80.5	14.6
	10	78.2	16.9	75.8	10.9	76.7	17.3
	20	77.0	16.3	80.0	18.3	83.0	21.4
Double cotton swab	1	86.5	9.5	93.6	8.7	94.2	7.5
	10	84.8	7.7	91.3	4.7	95.7	5.1
	20	88.0	6.6	90.8	6.1	96.7	5.5

[a] <Limit of detection (0.2 μg).

The overall procedure can be used as a part of a cleaning validation program in the acetylsalicylic pharmaceutical manufacturing.

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