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Development and validation of a flow-injection method for the determination of albumin tannate, the active component of a pharmaceutical preparation

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Abstract

A flow-injection analysis method for the determination of albumin tannate in tablets is reported. After optimization of the variables involved, the method has been characterized and validated in terms of calibration using three procedures: repeatability and reproducibility; ruggedness; and selectivity. Finally, it has been applied to real samples (tablets).

Keywords: Albumin tannate; Flow injection; Pharmaceuticals; Spectrophotometry

1. Introduction

Albumin tannate, a compound of tannic acid (tannin) with albumin (50%), is given by mouth for its astringent properties in the treatment of diarrhoea. It is stated to liberate tannic acid in the gastro-intestinal tract.

Tannins are phenolic polymers (molecular weight 500–3000 Da) which, through hydrogen bonding with peptide linkages, precipitate proteins from aqueous solutions, rendering plant proteins and reducing the activity of enzymes. Tannins are divided into four groups: (1) the hydrolyzable tannins, which are esters of glucose with gallic, *m*-digallic, or hexahydroxydiphenic acids; (2) proanthocyanidins, yielded by condensation of flavan-3-ols, that produce catechin, epicatechin and gallocatechin; (3) oxytannins, formed upon injury to the plants by oxidation of the catechins; and (4) β-tannins, a diverse group of compounds of lower molecular weight than those of the other categories, and capable of precipitating proteins [1]. A characteristic of this last group is its ability to precipitate with albumin, which facilitates its use as a component of pharmaceuticals for treatment of diarrhoea.

Tannins are frequently determined in wine, oil, tea, beer, food, and animal and vegetable tissues, but not in drugs. Therefore, no methods for the determination of albumin tannate in pharmaceuti-

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cal preparations are found in the recent literature, and very few methods appear for the determination of tannins [2]. Thus, this gap in the literature calls for the urgent development of a method for the determination of albumin tannate (or tannins) which could be included in the pharmacopoeias, as the existence of a validated method for the determination of an active compound is mandatory in order for a new pharmaceutical to be accepted by the relevant pharmacopoeia.

The aim of this work is the development and validation of a method for the determination of tannate in a new pharmaceutical product, tablets for the treatment of diarrhoea, which contains albumin tannate as active compound (504.00 mg), and Mingtai (microcrystalline cellulose M-102, 200.00 mg), Explotab (starch sodium glycolate, 37.50 mg), Xyloid (silica gel, 1.00 mg) and Stearine L2 SM (7.50 mg) as excipients per 750.00 mg tablet.

An automated method, based on the use of flow-injection anlaysis (FIA), that allows albumin tannate to be determined with no interference from excipients has been developed. The method is based on the reaction of tannic acid with the Folin–Ciocalteu phenol reagent in a basic medium, and monitoring of the coloured product at 760 nm. As stated in the literature, this is the most frequently used method for the determination of these compounds [1] which in addition can be easily automated [3]. The albumin tannate is hydrolyzed in a basic medium, and no previous separation of albumin is necessary to carry out the determination.

2. Experimental

2.1. Materials and reagents

All materials and reagents were of analytical grade and were used without further purification. Ultrapure water was used throughout.

A 100 mg l^{-1} stock solution of tannic acid (Sigma) in water was used. Working solutions were prepared by appropriate dilution of the stock solution, as required. Aqueous solutions of albumin tannate (Calmante Vitaminado S.A., Spain) of appropriate concentration in 2.5% ammonia (Panreac) were prepared daily. A 25% stock solution of Folin–Ciocalteu reagent (Merck) in water and a 1 M stock solution of sodium hydroxide (Merck) in water were also used.

2.2. Instruments and apparatus

A Gilson Minipuls-2 peristaltic pump, an Omnifit low-pressure injection valve, a Unicam 8625 spectrophotometer wiht a Hellma 178.QS flow-cell (18 μl inner volume), a Knaouer recorder and Teflon tubing (0.5 mm i.d.) were used to build the flow manifold.

A Bandelin Sonorex K 52 ultrasonic bath was used to dissolve the samples.

2.3. FIA conditions

The FI conditions were those found to be optimal from study of the variables. Their values are listed in Table 1.

2.4. Preparation of the standard solutions

Calibration solutions were prepared from a stock standard solution containing 50 mg l^{-1} albumin tannate in 2.5% ammonia in water. The solutions were sonicated for 3 min and kept in a refrigerator for 1 h, protected from light.

Table 1
Study of the variables

Variables	Range of study	Optimum value
Chemical variables		
NaOH (M)	0.3–2	1
F–C (%)	5–35	25
Ammonia (%)	1–10	2.5
FIA variables		
L1 (cm)		30
L2 (cm)	60–400	200
Injection volume (μl)	50–1000	275

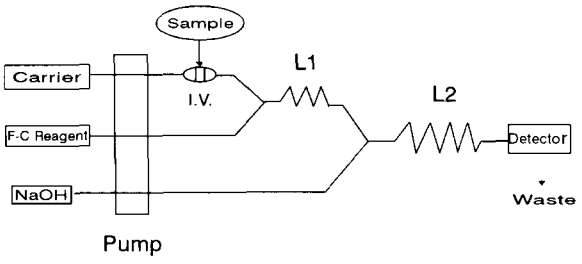

Fig. 1. FI manifold for the spectrophotometric determination of albumin tannate in pharmaceuticals: F–C, Folin–Ciocalteau; IV, injection valve; L, reactor.

2.5. Preparation of the sample solutions

The tablets, accurately weighed, were ground and mixed in a mortar. 5 mg of the powder was transferred to a 100 ml volumetric flask with 2.5% ammonia in water and sonicated for 3 min. The solution was kept in a refrigerator for 1 h, protected from light.

2.6. Procedure

The manifold in Fig. 1 is used as follows: 275 μl of the aqueous sample solution is injected into a carrier of ultrapure water, which merges with the Folin–Ciocalteou (F–C) reagent and then with a 1 M NaOH solution. The reaction product is formed along reactor L2 and is monitored at 760 nm on passage through the flow-cell. The flow rate of each channel was 1 ml min^{-1}.

3. Results and discussion

The variables affecting the system were classified into: (i) chemical; (ii) those characteristic of the dynamic manifold; and were optimized by the univariate method.

Direct injection of the sample into the reagent was checked for simplification of the FI manifold. Once again, higher sensitivity was obtained by homogeneous mixing at a confluence or merging point (see Fig. 1) with respect to dispersion of the sample into the carrier reagent.

When not stated, a 100 μg ml^{-1} standard solution of tannic acid was used as sample and 50 μl of this solution was injected for the optimization study. After this the optimum value found was used.

3.1. Optimization of variables

3.1.1. Chemical variables

The range over which these variables were studied and the optimal values found in terms of both sensitivity (height of the transient signal) and reproducibility are shown in Table 1.

3.1.2. FI variables

The optimal values of the chemical variables were used in performing this study. An alternative procedure consisting of exchanging the F–C and NaOH streams was tested in order to improve sensitivity. Ill-defined peaks and lower absorbance at the maximum were obtained so the manifold shown in Fig. 1 was used. The only function of reactor L1 was to homogenize the mixture from the sample and F–C channels and so a length of 30 cm was sufficient for this purpose. The length of L2 determined the extent of the derivatization reaction once a flow rate of 1 ml min^{-1} was established. A length of 200 cm was found to be optimal as shorter values did not allow sufficient development of the derivatization reaction, while longer lengths decreased the peak height as the contribution of the dispersion surpassed that of the reaction development at that residence time. An injection volume of 275 μl was selected as optimum. Above this value the increase in sensitivity was very slight and did not compensate for the decrease in sampling frequency.

3.2. Stability of the sample solutions

The influence of temperature, time and light on the stability of the solutions of albumin tannate was tested. The absorbance of the solutions remained constant for $\approx$3 h in a refrigerator or in an ice bath. The presence of light affected the measurements for the first 5 min after preparing the solution of albumin tannate, but had no effect after this period. The temperature of the reagents and carriers had no significant effect on the stability as the residence time was only 20 s.

Table 2
Numerical values of the SC, AC and YC parameters

Group[a]	Parameter	SC	AC	YC
I	n	18	4	4
	a	-2.22×10^{-4}	0.129	1.50×10^{-3}
	b	8.93×10^{-3}	8.73×10^{-3}	6.22×10^{-3}
	s	2.35×10^{-3}	2.86×10^{-3}	9.49×10^{-3}
	r^2 (%)	99.98	99.96	99.99
II	S_p	2.42×10^{-3}		
	t(b)[b]	1.740 (critical value = 2.878)		
	b_p	8.91×10^{-3}		
	a'	2.05×10^{-4}	0.127	
	Yb[c]			1.29×10^{-3}

[a] I: from experimental measurements; II: new corrected values calculated from Ref. [4].
[b] Significant level = 1%.
[c] Youden blank.

3.3. Validation of the method

3.3.1. Calibration

The standard addition methodology [4] was used for calibration. The sets of data obtained in three calibration experiments [namely: with standard solutions, standard calibration (SC); with standard additions, standard-addition calibration (AC); and with portions of sample, Youden calibration (YC)] were used. The accuracy of the analytical results was checked by comparing both the analyte contents in the different calibrations and the recoveries, calculated by dividing the net content found by that added for each addition. The ALAMIN program was used for calculations [5]. The SC was run with triplicate injections of the standard solutions and the response vs. concentration curve was linear in the range 0–50 mg l^{-1}. Only one injection of each solution was made in the two other calibration procedures. The numerical values of the parameters of these calibrations are shown in Table 2. The Student t-test shows the similarity of the SC and AC slopes. Also, the intercepts of the SC and YC curves are the same.

The analyte contents in the test solutions, as obtained from the values in Table 2, are as follows:

SC method: 14.20 mg l^{-1}

AC method: 14.04 mg l^{-1}

$t = 1.039$ (critical value = 2.093)

The results from SC and AC are not significantly different, so the method is accurate. The average recovery (Table 3) is 100%. This supports the accuracy of the method. The conclusion obtained is that the determination of albumin tannate in tablets can be carried out directly by the SC method. The figures of merit of the method, calculated from the SC data set [6], are shown in Table 4.

3.3.2. Sample throughput

The sampling frequency under the optimal working conditions was 50 h^{-1}.

3.3.3. Repeatability

The method was applied to 10 standard solutions (25 mg l^{-1}) of albumin tannate under the

Table 3
Results of recovery assays to check accuracy

Concentration added (mg l^{-1})		Recovery (%)[a]
Standard analyte	Sample	
10	20	101.0
20	20	101.0
30	20	98.0

[a] Average recovery: 100.0%.

Table 4
Features of the analytical method from standard calibration data set

Linearity	99.64
Analytical sensitivity (mg l^{-1} of albumin tannate)	0.26
Detection limit (mg l^{-1} of albumin tannate)	0.56
Determination limit (mg l^{-1} of albumin tannate)	1.88
Precision (relative standard deviation (%), at different concentrations expressed in mg l^{-1}):	
10	1.71
20	0.83
30	0.55
40	0.43
50	0.38

optimal working conditions. All the measurements were made the same day, under identical experimental conditions and by three triplicate injections of each solution. The results of this study are as follows:

Average absorbance = 0.226 a.u.

RSD = 1.17%

3.3.4. Reproducibility

The method was also applied to 10 standard soultions (25 mg l^{-1}) of albumin tannate, under the optimal working conditions, on different days, injecting each solution in triplicate. The results obtained are:

Average absorbance = 0.223 a.u.

RSD = 1.76%

3.3.5. Ruggedness

A study of the effects on the predicted response of a variation of ±5% in the optimal values of the concentrations of F–C, NaOH and ammonia, and of ±10% on the flow rates of F–C, NaOH and carrier was performed [7]. A Plackett–Burman two-levels design was selected for this study by inserting one "dummy" variable to evaluate the potential interactions. The value of the standard deviation (s) of the predicted analytical signal for the tested concentration level was used as an external significance criterion in all statistical tests. The results of this study showed that the method is rugged for the concentrations of F–C, NaOH and ammonia, but not for the flow rate (significance level $\alpha = 0.05$). All the calculations were made using the STATGRAPHICS program [8].

3.3.6. Selectivity

The selectivity of the method was checked by monitoring a standard solution of albumin tannate in the presence of the other components of the tablets (excipients), at the same concentration levels as for the tablets. The response was not different from that obtained in the calibration. The absorbance values of solutions of the excipients alone (at 760 nm) were measured too, showing no significant difference from the baseline; the excipients caused no effect so that determination of the active compound of this pharmaceutical is free from interferences.

3.4. Application of the method to real samples

The performance of the method was tested by applying it to the determination of albumin tannate in 11 samples of powder obtained from 85 commercial tablets and applying the "quarter method". Samples were prepared as described previously and injected in triplicate. The final result was: concentration = 515.9 ± 1.3 mg albumin tannate per tablet.

The deviation of the mean value is acceptable and is consistent with those calculated in the study of the precision (RSD = 1.17% and 1.76% for the repeatability and reproducibility studies respectively).

4. Conclusions

A semi-automated method for the determination of the active compound (albumin tannate) in a new pharmaceutical has been developed with a double aim, namely:

(i) to propose the acceptance of this product by the Spanish Pharmacopoeia based on a reliable method for the determination/validation of its active component;

(ii) to endow Pharmacopoeia with a reliable method for the determination of this drug.

The introduction of a semi-automated continuous method in the Pharmacopoeia could be the starting point for updating the conventional, old-fashioned methods accepted at present by pharmacopoeias.

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