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In vitro release of valerenic and hydroxyvalerenic acids from valerian tablets

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Although most commercial valerian formulations are coated tablets not any comparison study of their drug release profiles has been published so far. The main objective of this work is to establish a drug release test suitable for studying and comparing different valerian tablets. Thus, hydroxyvalerenic and valerenic acid concentrations were assayed by HPLC using a C_{18} Kromasil (200 × 4.6 mm, 5 μm) column and a mobile phase containing methanol and an orthophosphoric acid solution 0.5% v/v in water at a ratio of 75 : 25 at a constant flow rate of 1 ml/min. Saturation solubilities for hydroxyvalerenic and valerenic acid at pH 6.8 were 26 ± 5.1 and 1 ± 0.6 μg/ml, respectively. Usually for drugs with such low solubility values, their oral absorption and hence bioavailability are limited by their dissolution characteristics. A dissolution test was conducted according to the general method 2 (paddles) of USP 24 using 500 ml buffer medium (pH 6.8) at 50 rpm. Five different formulations were studied and compared: one uncoated tablet formulation and four marketed coated tablets. The uncoated tablet formulation had the fastest release profile, whereas the coated tablets manifested very different release patterns, depending on the type of formulation. Because of these differences in drug release pattern not every tablet formulation may be appropriate for the same clinical indications. Clinical data are required to confirm the correlation between drug release pattern and the therapeutically value of each formulation.

1. Introduction

Valerian drugs are well known for their sedative therapeutic effects. Due to their unpleasant smell they are usually marketed as coated tablets. Depending on the type and amount of coating agents, different drug release rates can be achieved. Anxiolytic and hypnotic drugs, such as the valerian products, require an immediate release profile. For low soluble drugs, the rate of release is sometimes the limiting factor for oral absorption [1]. As far as we know, there is no documented study on the drug release characteristics of marketed valerian tablets.

The aim of this work was to propose a dissolution test and to compare the rate of drug release from different tablet formulations. The release rate tests are used as *in vitro* equivalence indicators, among drugs with the same active ingredients. Although these results are obviously not as conclusive as *in vivo* bioequivalence trials, they are very useful in quality control and hence, they are generally included in many pharmacopoeias. In order to carry out the valerian tablets release test, it is necessary to conduct a quantitative HPLC analysis. Although there are numerous active components in the valerian tablets, only hydroxyvalerenic and valerenic acids have been assayed in this work, due to the limited availability of HPLC reference standards.

2. Investigations, results and discussion

Fig. 1A shows a HPLC chromatogram of the standard hydroxyvalerenic and valerenic acids used as reference products. Fig. 1B shows an chromatogram of valerian root sample test from a tablet. The HPLC of valerian root sample shows several peaks, which can be related to other sample components such as acetoxyvalerenic acid or valerenal. Some of these products have pharmacological effects, but they are not commercially available as standard reference products for HPLC assay. A great variability on hydroxyvalerenic and valerenic acid content in the different formulations has been found and the results are shown in Table 1. Similar results have been reported previously [2–4]. Most of these differences can be due to the natural variability in the plant contents depending on the source, time of collection and extraction procedures. If not standardized, this variability may be manifested not only among different manufacturers, but also among different batches of the same manufacturer.

Table 2 shows the solubility coefficients of hydroxyvalerenic and valerenic acids in different media. Even though hydroxyvalerenic acid is apparently more soluble than valerenic acid under the three different experiment conditions, both of them, according to the European Pharmacopoeia criteria, can be classified as practically insoluble materials

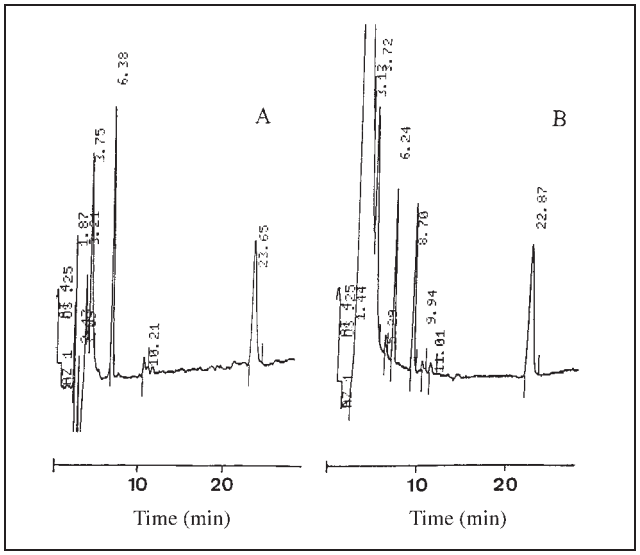

Fig. 1: HPLC chromatograms of standard hydroxyvalerenic and valerenic acid reference samples (A) and a problem sample from a tablet (B).

[5]. The effect of pH on solubility of both products, is due to the medium ionisation property; and therefore, at neutral pH, the solubility of acidic substances like (hydroxyvalerenic and valerenic acids) tends to be higher than at acidic pH. Normally, for poorly water soluble drugs the oral absorption is limited by drug dissolution rate, which can be considered as the rate-limiting step [1].

According to the solubility data available, an acidic pH, such as that present in the stomach, can result in very low dissolution rates of valerenic and hydroxyvalerenic acids. Moreover, since acetoxyvalerenic is hydrolysed to hydroxyvalerenic acid, this hinders its accurate estimation during the dissolution study. Furthermore, some of the marketed coated tables are enteric formulations, and therefore, there is no or very low drug release, which is usually below the detection limits for the HPLC. Thus, the dissolution study has been focused on pH 6.8, which may probably be the suitable pH for the hydroxyvalerenic and valerenic acids released after dissolution, and later on absorbed. Hydroxyvalerenic and valerenic acid released from one uncoated tablet and four marketed coated tablets formulations are shown in Fig. 2 and 3, respectively.

Table 1: Composition of the different studied tablet formulations (µg/tablet)

Formulation	Declaration of manufacturer	Hydroxyvalerenic acid (µg)	Valerenic acid (µg)
A	200 mg of dried root powder of *V. officinalis*	12.2 ± 1.2	73.4 ± 2.6
B	Dried extract equivalent to 560 mg of dried root powder of *V. officinalis*	10.1 ± 1.2	24.3 ± 1
C	45 mg of dried extract of *V. officinalis*	14.8 ± 1.4	28.1 ± 1.5
D	200 mg of dried root powder of *V. officinalis*	28.1 ± 0.5	109 ± 2.2
E	100 mg of dried root powder of *V. officinalis*	14.9 ± 1.5	69.1 ± 2.4

Mean results and standard deviation of three experiments.

Table 2: Saturation solubility of C_s hydroxyvalerenic and valerenic acid in different media

Dissolution medium	C_s (µg/ml) Hydroxyvalerenic acid	C_s (µg/ml) Valerenic acid
Deionised water	20.6 ± 3.9	0.4 ± 0.1
1.2 pH medium	8.7 ± 2.9	0.3 ± 0.1
6.8 pH medium	26 ± 5.1	1 ± 0.6

Mean results and standard deviations of three experiments.

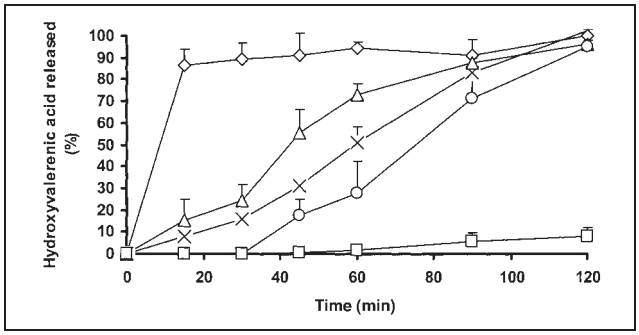

Fig. 2: Hydroxyvalerenic acid release profile of five different formulations in 6.8 pH medium. Key: ◇-Formulation A, ×-Formulation B, △-Formulation C, ○-Formulation D and □-Formulation E. (Mean value and standard deviation of six experiments)

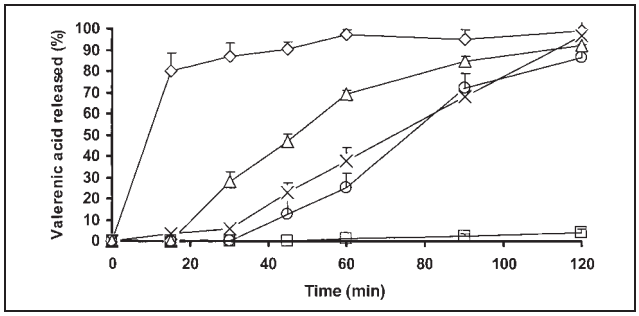

Fig. 3: Valerenic acid release profile of five different formulations in 6.8 pH medium. Key: ◇-Formulation A, ×-Formulation B, △-Formulation C, ○-Formulation D and □-Formulation E. (Mean value and standard deviation of six experiments)

Obviously, the uncoated tablet formulation (formulation A) shows the fastest drug release profile, while the marketed tablets have a different release pattern depending on type of formulation. Formulations B, C and D have very similar drug release profiles, which are quite different from formulation E. These *in vitro* differences between formulations can hardly be extrapolated to *in vivo* conditions. According to the United States Pharmacopoeia [6] "dissolution tests are the discriminating formulation factors, . . . , that it is not uncommon for a clinically acceptable article to perform poorly in a typical dissolution test". Similarly, for other drugs, such as metoprolol tartrate, even though the *in vitro* differences on the drug release have been observed among different formulations, not even a single bioequivalence problem has been reported [7]. Furthermore, it is important to point out that drug release has been expressed as percentage of hydroxyvalerenic and valerenic acids present in the formulation, and according to the data in Table 1, there are important differences in contents among formulations.

The results obtained after 120 min of test were analysed by an ANOVA test (Statgraphics 4.0 program, Manugistics). Valerenic acid data are shown in Table 3. Statistically significant differences were found among the formulations, except between B and C. Similar results were found for the hydroxyvalerenic acid data (not shown).

Another problem to be taken into consideration is that only hydroxyvalerenic and valerenic acids have been studied, and many other active components in the valerian tablets have not been quantified [8, 9]. For that matter, it is necessary to express the drug content of each of these

Table 3: One way ANOVA (F-ratio 156.4) comparative study

Formulations	A	B	C	D	E
A	×	S^{***}	S^{***}	S^{***}	S^{***}
B	–	×	NS	S^{***}	S^{**}
C	–	–	×	S^{***}	S^{**}
D	–	–	–	×	S^{***}
E	–	–	–	–	×

Amount (μg/tablet) of valerenic acid released after 120 minutes for the five different formulations. Key: NS: difference not statistically significant ($P > 0.05$), S^{**} difference statistically significant ($P \leq 0.01$), S^{***} difference statistically significant ($P \leq 0.001$).

products in a different way from the existing one, since most of them are composed of root powder or extract.

Hence, to be completely assured of the *in vivo* equivalence among the different formulations, a bioequivalence study is recommended. But again, the problem is which one of the valerian components should be quantified and which analytical method can be sensitive enough to study such low concentrations in the biological samples. In addition to that, it is generally recognised that pharmacokinetic studies on herbal drugs are more difficult to be carried out than on conventional drugs [10]. However, Wagner and Jurcic [11] conducted, a pharmacokinetic study on valerian products, by means of radioactive assay.

Finally, one important aspect about this drug is that depending on the rate of drug release, various marketed formulations of valerian tablets can be classified into immediate or slow release formulations. This classification can be a useful tool for prescription, because drug release, oral absorption and duration of drug action for these formulations, are all interrelated. For example, when valerian products are prescribed as hypnotics for patients with difficulty in finding sleep, then an immediate release formulation might be the best option. Contrary, for insomniac people, whose main problem is to wake up early, probably a slow release formulation can be a better option than a fast release product.

In conclusion, an easy and simple *in vitro* dissolution test for valerian tablet-drug release study is proposed and used for a comparative purpose. And, the observed differences in the rate of drug release among the valerian marketed tablets, have been clearly depicted.

3. Experimental

3.1. Materials

HPLC reference standards for hydroxyvalerenic and valerenic acids were purchased from Extrasynthese (France). Then the following five valerian tablet formulations were used; one uncoated tablet formulation was prepared by mixing valerian root powder, microcrystalline cellulose, Emcompress® and magnesium stearate at the proportions 20 : 15 : 15 : 0.5 (w : w) and then tableted in an excentric tablet press with flat punches of 14 mm diameter in order to obtain tablets of a mean weight of 505 mg and a disintegration time of less than 15 min. The valerian root powder was purchased from Roig Farma S.A. (Barcelona, Spain). These uncoated tablets are referred as formulation A. Four commercial coated valerian tablet formulations were also studied: Cirkused® from Roha Arzneimittel GmbH (batch M 1), Valdispert® from Solvay Pharma S.A. (batch M 8), Kneipp® from Fher S.A. (batch J 120) and Valeriana-Leo from Byk Leo (batch J 27). In this work, these formulations will be referred as B, C, D and E formulations. All other chemicals are in accordance with analytical standard from either Merck or Panreac.

3.2. HPLC assay

Analytical chromatography was performed in a modular liquid chromatograph equipped with a Gilson 305 isocratic pump, an automatic sampler (Gilson 231 XL) fitted to a 100 μl sampling loop (Rheodyne), a variable wavelength detector (Gilson 116) and an integrator (Spectra-Physics SP-270). A method similar to that proposed by Hänsel and Schulz [12] has been used. A constant flow of 1 ml/min of a mobile phase containing methanol and an orthophosphoric acid solution 0.5% v/v in water at a proportion 75 : 25 v/v was employed. The analysis was conducted in a Kromasil 100 ODS2 column (200 × 4.6 mm, 5 μm) from Teknokroma (Barcelona, Spain). The samples were measured at 225 nm and data were recorded on an integrator at attenuation 4. Under these experimental conditions, hydroxyvalerenic and valerenic acid had retention times of approximately 6 and 23 min, respectively. The linearity of the method was studied at the range between 0.1 and 1 μg/ml ($n = 7$). The results were analysed by linear regression and the obtained correlation coefficients were approximately 0.99. The quantitation limit was defined as the lowest concentration which give rise to a signal capable of being quantified by the integrator (signal-to-noise-ratio=5), which were found to be 0.01 and 0.02 μg/ml for hydroxyvalerenic and valerenic acid, respectively. Inter-day precision during three consecutive days was 1 and 1.2%, respectively.

3.3. Hydroxyvalerenic and valerenic acid content in tablets

Five individual tablets were weighed and then milled in a mortar. An exact amount of approximately 100 mg of the powder was dissolved in 50 ml of ethanol 70%. Then it was sonicated for 60 min in an ultrasonic bath (Branson 2200, Bransonic, USA), filtered through 0.45 μm and then assayed by HPLC.

3.4. Solubility study

Aqueous saturation solubility of hydroxyvalerenic and valerenic acid from a valerian root powder (*V. officinalis* from Roig Farma S.A.) was studied in the following aqueous media: deionized water; approximately 0.1 N solution of diluted HCl acid at pH 1.2 and buffer solution (0.005M KH_2PO_4 with 0.1 N NaOH adjusted up to pH 6.8). All these samples were studied in an excess of valerian powder which was maintained at 37 °C for three days under mechanical stirring. Then, samples were filtered, diluted and assayed by HPLC.

3.5. Drug release test

Hydroxyvalerenic and valerenic acid release from the five different tablet formulations were studied in a Vankel (USA) dissolution test equipment according to the general method 2 (paddle) of USP 24 (2000) for solid dosage forms. In each vessel, 1 to 5 valerenic acid tablets were used, depending on its content in that particular formulation. The maximum concentration in each vessel was lower than 0.5 μg/ml. The dissolution medium for each vessel was composed of 500 ml (0.005M KH_2PO_4 with 0.1 N NaOH) buffer at pH 6.8. The temperature was kept at 37 °C, at the stirring speed of 50 rpm. The sampling was carried out at 15, 30, 45, 60, 90 and 120 min; and all samples were immediately filtered and then assayed by HPLC.

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