

INFLUENCE OF EXTRACTION SOLVENT ON THE ERYTHROMYCIN ETHYLSUCCINATE SEPARATION FROM ORAL SUSPENDABLE POWDER

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Abstract

The aim of this work was to carry out a separation of erythromycin ethylsuccinate from oral dry suspensions, which don't correspond at batch quality control. The oral dry suspensions contain erythromycin ethylsuccinate, sugar, sodium saccharin, preservatives, flavours and sodium citrate. We recovered the active substance from the powder mixture, mainly by a solid-liquid extraction process followed by the crystallization of erythromycin ethylsuccinate with a non-solvent. The extraction solvents used in this study were: acetone, ethanol, chloroform and methanol. As non-solvent for crystallization we used distilled water in different volumes. The best results were obtained when acetone was used as extraction solvent and water as non-solvent for crystallization (1:4). The obtained erythromycin ethylsuccinate corresponded from the purity and activity point of view with erythromycin national standard and the method is more convenient from the obtained quantity of erythromycin ethylsuccinate, output and economic points of view.

Rezumat

Scopul acestei lucrări este separarea etilsuccinatului de eritromicină din pulberi suspendabile orale, ce nu corespund la controlul de calitate. Pulberea orală suspendabilă conține etilsuccinat de eritromicină, zahăr, zaharină sodică, conservanți, aromatizanți, citrat de sodiu. Am recuperat etilsuccinatul de eritromicină din amestecul de pulberi printr-o extracție solid-lichid urmată de cristalizarea etilsuccinatului de eritromicină cu ajutorul unui non-solvent. În acest studiu am utilizat ca solvenți de extracție: acetona, etanolul, metanolul, cloroformul, iar ca non-solvent apa distilată în proporții diferite. Cele mai bune rezultate s-au obținut atunci când s-a utilizat acetona ca solvent de extracție și apa distilată ca non-solvent în proporție de 1:4. Etilsuccinatul de eritromicină astfel recuperat corespunde din punct de vedere al purității și al activității microbiologice cu standardul național de eritromicină. Metoda utilizată este economică, are randament mare, iar pierderile de substanță activă sunt mici.

Keywords: erythromycin esters, erythromycin ethylsuccinate, extraction, oral suspensions

Introduction

Erythromycin is a macrolide antibiotic drug, with an antimicrobial spectrum similar to penicillin. It is used by patients who have an allergy to penicillins under the ester or salt forms [2, 6]. Erythromycin ethylsuccinate (E.E.) is an ester prodrug of erythromycin that is administered as oral dry suspensions or tablets. E.E. is produced by S.C. "Antibiotice" S.A. as vials with oral dry suspensions [5, 7]. A vial contains 26 grams of powder that possesses 2.4 g of base erythromycin corresponding to 3.1 g of erythromycin ethylsuccinate, sugar (~22 g), sodium saccharin, preservatives (nipagine-nipasole ~ 1 g), flavours and sodium citrate.

In some situations, at batch quality control, the powder mixture doesn't correspond because there are some mistaken weighings, physical and microbiological contaminations. In these situations it is necessary to recover E.E. from the powder mixture, considering the high price of the ester (~ 120 U. S. dollars/kg) [1, 3, 4].

In order to do this a solid-liquid extraction process was performed, followed by the crystallization of the E.E. with a non-solvent.

Materials and methods

Acetone (p.a.), ethanol (p.a.), chloroform (p.a.), methanol (p.a.) and distilled water were used as solvents.

Erythromycin ethylsuccinate (E.E.) national standard (n.s.), erythromycin n.s. with an activity of 1:937 U and 2.02 % humidity, the standard solutions being prepared in methanol (p.a.) were used.

The *Micrococcus luteus* ATCC 9341 was used as test microorganism.

The E.E. solubility was studied in the solvents used in this study. The determinations were performed according to Xth Romanian Pharmacopoeia (R.Ph.X) at 20±2⁰C. R.Ph.X stipulates that the substance should be freely soluble if 1 g of tested substance dissolved in a quantity of solvent between 1 mL and 10 mL.

A sample of oral dry suspension (26 g), containing the active substance and excipients, was dissolved in 60 mL of acetone, ethanol, chloroform and methanol, respectively. The sugar, sodium saccharin, the flavours and sodium citrate, which are insoluble in these solvents, were filtered and the operation was repeated. The reunited filtrates (120 mL) were evaporated on a water-bath until the first crystals appeared, when in the evaporating dish remained about 30 mL of concentrate. After that, 120 mL of cold distilled water, as a non-solvent, were added. Through cooling on an ice-bath E.E. was crystallized, then filtered and the precipitate was washed with

cold distilled water and dried in an air drift at room temperature. There were used four variants of the method (method I, II, III, IV), using different solvents.

After we established that the best solvent for solid-liquid extraction is acetone, we applied three variants of this method using different volumes of distilled water (as non-solvent) according to the concentration degree of the filtrates at their evaporation on the water-bath. In the first variant (variant 1) we evaporated on water-bath at half of the volume and there were added 240 mL of distilled water. In the second variant (variant 2) we evaporated until the first crystals appeared and there were added 120 mL of distilled water. In the third variant (variant 3) we evaporated on the water bath up to the dry point and there were added 60 mL of distilled water.

The quality control of the obtained E.E. was performed according to the Erythromycin ethylsuccinate monograph from Xth Romanian Pharmacopoeia (R.Ph.X) and 6th European Pharmacopoeia (Ph.Eur.).

For the qualitative determination we used thin-layer chromatography with silica gel G as the coating substance and a mixture of solvents as mobile phase. The E.E. corresponding spots of the samples must be similar regarding the position, color and diameter to that of E.E. n.s. The erythromycin corresponding spots of the samples must be similar in position, color and diameter to that of erythromycin n.s. The monograph stipulates that erythromycin within the E.E. is no more than 5% [8, 9].

The quantitative determination was achieved by a microbiological assay through the agar diffusion method of the E.E. [8, 9]. The microbiological activity of the E.E. samples was compared with the erythromycin national standard activity (1:937 U and 2.02 % humidity). The samples correspond if the microbiological activity is at least 90.0% and no more than 110.0% of the microbiological activity of erythromycin national standard.

Results and discussion

The solubility determination revealed that in all tested solvents erythromycin ethylsuccinate (E.E.) is freely soluble, but in acetone and chloroform is more soluble, as it can be seen in table I.

Table I
The erythromycin ethylsuccinate solubility in some solvents

No.	Solvent	Volume of solvent for dissolution of 1 g E.E. (mL)
1.	Acetone	2
2.	Ethanol	9
3.	Methanol	4
4.	Chloroform	2

During the E.E. crystallization from the mixture of oral dry suspension, it could be noticed that the highest quantity of the E.E. (2.77 g) was obtained when acetone was used as extraction solvent (table II). Smaller quantities were obtained when chloroform, respectively methanol was used and the smallest quantities were obtained when ethanol was used. Regarding the obtained quantities of E.E. we observed that the greatest yield (89.35%) was registered when acetone was used as extraction solvent (table II).

By dissolving the sample in these solvents the sugar remained in suspension and was filtered. In 26 g of sample there are ~ 22 g of sugar, so we weighted the obtained sugar precipitates to verify the extraction. The greatest quantity of removed sugar was obtained when we used acetone as extraction solvent (table II).

Table II

The obtained amounts of erythromycin ethylsuccinate, removed sugar and yields using different solvents

Method and used solvent	Amounts of E.E. (g)				Amounts of eliminated sugar (g)				Yield (%)
	1 st det.*	2 nd det.	3 rd det.	Average of det.	1 st det.	2 nd det.	3 rd det.	Average of det.	
I Acetone	2.88	2.62	2.81	2.77	22.1	21.9	22	22	89.35
II Ethanol	1.98	2.34	2.04	2.12	19.9	20.2	21.7	20.6	68.38
III Methanol	2.31	2.08	2.18	2.19	21.8	21.1	21.3	21.4	70.64
IV Chloroform	2.64	2.76	2.37	2.59	22	22	21.7	21.9	83.54

*det. = determination

It can be noticed (as seen in table III) that the smallest quantity of distilled water used as non-solvent was that of variant 3 (60 mL), but the quantity of obtained E.E. was small (1.85 g) and the yield as well (59.67%). The purification by the 2nd variant is more advantageous, since a greater quantity of E.E. was retrieved (2.78 g) and a greater yield was achieved (89.67%).

The qualitative determination shows that in all variants the colour, position and diameter of the erythromycin corresponding spots were not greater than the erythromycin n.s., therefore the erythromycin within the samples was not higher than 5%. The E.E. corresponding spots had the same

colour, position and diameter as the E.E. n.s. spot. The values of the Rf were calculated ($R_f=6.5/11.5$, $R_f=0.56$), therefore the samples contain E.E.

Table III

The distilled water volumes used as non-solvent and the obtained amounts of erythromycin ethylsuccinate (E.E.)

Variants with different volumes of water	Amounts of E.E. (g)				Yield (%)
	1 st det. *	2 nd det.	3 rd det.	Average of det.	
1 (240 mL)	2.43	2.27	2.59	2.43	78.38
2 (120 mL)	2.26	3.01	3.09	2.78	89.67
3 (60 mL)	0.94	2.77	1.84	1.85	59.67

*det. = determination

From the quantitative determination (table IV) it can be noticed that the obtained E.E. had a very good activity, especially the E.E. obtained through the 2nd variant (926 µg/mL and 98.8%), so the samples corresponded to the R.Ph.X and Ph.Eur requirements [8, 9].

Table IV

The purified E.E. activity

Variant	Activity EE (µg/mL)	Activity EE (%)
Erythromycin n.s.	937	100
1	925	98.7
2	926	98.8
3	922.8	98.48

Conclusions

The separation of erythromycin ethylsuccinate (E.E.) from a mixture of oral dry suspension can be performed by a solid-liquid extraction with acetone as extracting solvent (method I).

The E.E. crystallization can be performed using water as non-solvent. The separation is achieved by evaporating the acetone concentrate until the first crystals appear (variant 2), using 120 mL of water as non-solvent (1:4).

Working in such manner greater amounts of E.E. were obtained (2.78 g) and a greater yield was achieved (89.97%). The quantities of acetone and water used, as solvent and non-solvent respectively, in order to separate E.E. were the smallest ones, therefore the procedure is an economic one. Also, acetone has a lower boiling point and so its recovery is cheaper, which is an important factor from the industrial point of view. The obtained E.E. possesses a great purity and its microbiological activity is very good (98.8%).

Authors contribution

All authors had equivalent contribution to this article.

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