Binary Mixtures of Morphine and Furosemide: Compatibility and Stability at Different Concentrations

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ABSTRACT

Objectives: In order to avoid separate injections, admixtures of drugs are frequently used in palliative care settings. There are different factors that can influence the compatibility and stability of the mixture: drug type, concentration, solvent, container, temperature and light. There are some mixtures of drugs with proven stability, but there is lack of evidence about the stability and compatibility of the combination of morphine and furosemide. The purpose is to evaluate the compatibility and stability of two admixtures of morphine and furosemide at two different temperatures (25°C and 37°C). The concentrations of the admixtures are: 3.0 mg/mL-2.0 mg/mL; 1.0 mg/mL-0.6 mg/mL; in NaCl 0.9% stored in elastomeric infusors protected from light. **Methods:** The samples were prepared and diluted in NaCl 0.9% in elastomeric infusor in triplicate to obtain four different conditions of concentration and/or temperature of storage (concentration: 3.0 mg/mL-2.0 mg/mL, 1.0 mg/mL–0.6 mg/mL of morphine and furosemide respectively; temperature of storage 25°C and 37°C). The concentration of each constituent drug into different mixtures was periodically determined using a HPLC-UV method. The drugs were chromatographed on a C_{18} reverse phase column; the mobile phase was acetonitrile-water 40:60 (v/v); flow rate 1.5 mL/min. Morphine and furosemide concentrations were determined at 235 nm by interpolation from the calibration curves prepared at (0, 1, 2, 3, 7, 8, 9, 10, 14, 15) days from the standards. Statgraphics centurion XVI program has been used to data treatment. **Results:** The stability of the admixtures diluted in NaCl 0.9% are as follow: morphine-furosemide (3.0 mg/mL-2.0 mg/mL) is stable (retained > 95% of their initial concentration) eight days at 25°C and two day at 37°C; (1.0 mg/mL-0.6 mg/mL) is stable thirty days at 25°C and two day at 37°C. **Conclusion:** The admixture of morphine and furosemide in NaCl 0.9% in elastomeric infusor can be safely used in palliative care for at least two days. Concentrations of the admixture can be prepared in advance and stored at room temperature, but the infusion cannot be longer than two days.

Key words: Furosemide, HPLC, Mixtures, Morphine, Stability.

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INTRODUCTION

The definition of the World Health Organization (WHO)[1] about palliative care is follow: an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual. Palliative care provides relief from pain and other distressing symptoms affirms life and regards dying as a normal process, intends neither to hasten or postpone death, integrates the psychological and spiritual aspects of patient care, offers a support system to help patients live

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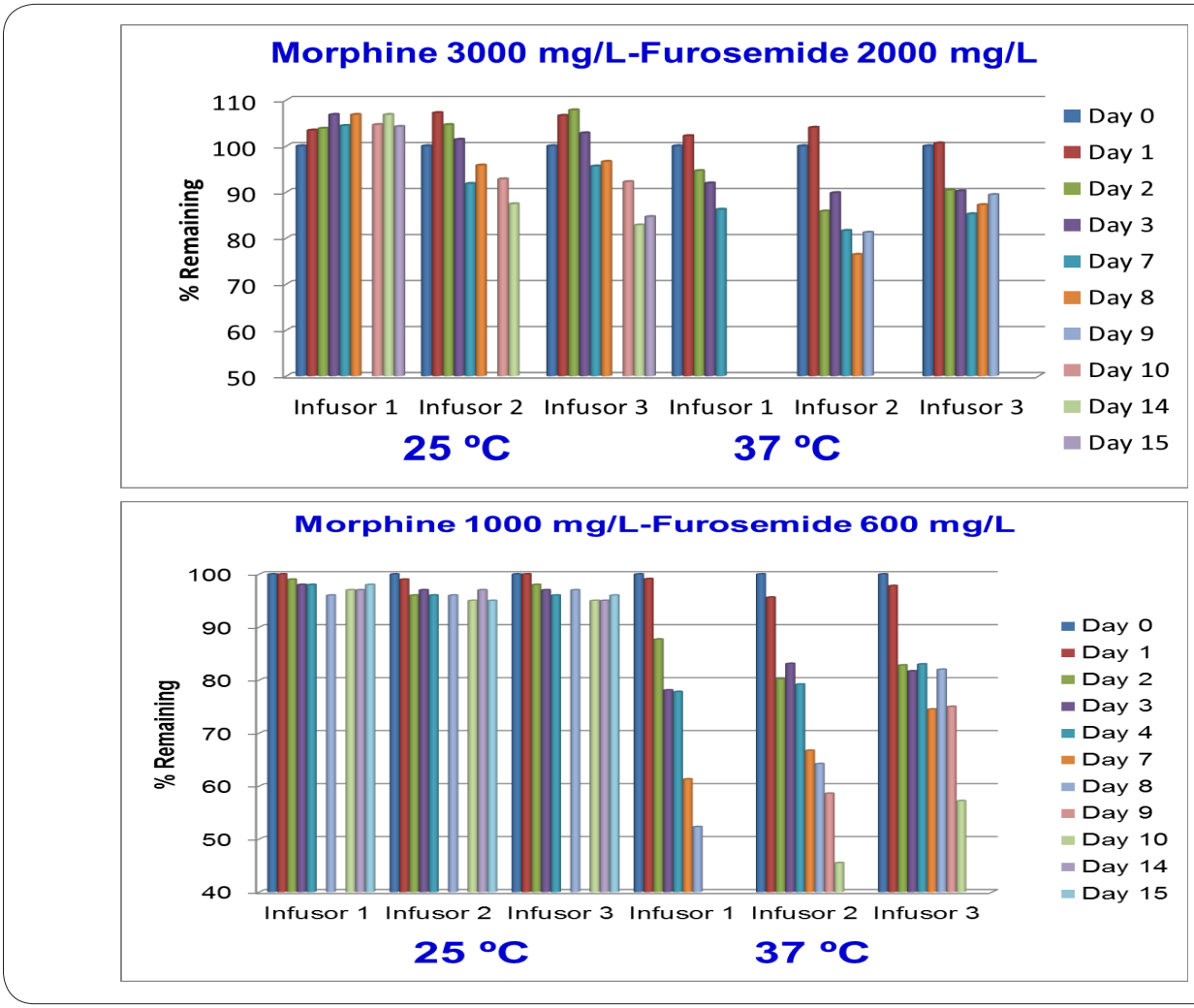

Graphical abstract

as actively as possible until death, offers a support system to help the family cope during the patients illness and in their own bereavement, uses a team approach to address the needs of patients and their families, including bereavement counselling, if indicated, will enhance quality of life, and may also positively influence the course of illness, is applicable early in the course of illness, in conjunction with other therapies that are intended to prolong life, such as chemotherapy or radiation therapy, and includes those investigations needed to better understand and manage distressing clinical complications.

To obtain optimal symptom control in these patients, the simultaneous administration of more than one drug is often required.[2]

When the oral administration of drugs is no longer possible at a later stage of the disease because symptoms worsen and the patient's general condition deteriorates, alternative methods of delivering the drugs may be necessary. The ideal system should deliver the drug reliably in a pain-free manner, use a minimum amount of nursing time and allow the patient remain mobile. In addition, it should be simple to use and not too costly.[3]

To avoid the use of different infusion needles, it may be beneficial to mix different drugs in one single infuser. Drug infusers offer the possibility of continuous subcutaneous drug administration that, compared to intermittent injections, gives a more constant plasma concentration. The continuous subcutaneous infusion of drugs has become an accepted practice, especially in the palliative care of cancer patients, in the 20 years.[4-6] The patients tolerate the treatment well, and infusion-site infection and abscess are rare.

Morphine is an opioid analgesic used for the treatment of moderate to severe pain. It is recommended by the WHO for the relief of moderate cancer-related pain. It is the opioid of choice in palliative and terminal care. Morphine is predominantly cleared from body by metabolism to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Furosemide is a loop diuretic, which is an anthranilic acid derivative (5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl)amino]

benzoic acid) used in the treatment of congestive heart failure and edema. His medication is also used to treat high blood pressure (hypertension). Furosemide works by blocking the absorption of salt and fluid in the kidney tubules, causing a profound increase in urine output (diuresis). The diuretic effect of furosemide can cause body water and electrolyte depletion. Therefore, careful medical supervision is necessary during treatment.

The physical compatibility and/or stability of several drugs in solution destined to subcutaneous infusion has been widely studied,[7-11] although in some studies only visual inspection of the samples was performed, providing information on the physical compatibility but not on the chemical stability of the drugs in the mixture. Moreover, studies have not yet been done regarding the combination of morphine and furosemide in solution in which the quantification of both drugs was performed by HPLC. On the other hand, patients will only benefit from the use of mixtures if those mixtures are of good quality since the administration of incompatible mixtures could cause irritation. Besides, chemical incompatibility and instability of the drugs in the mixture will result in an inadequate therapeutic outcome, and the degradation products may cause additional side effects.

Therefore, the aim of this study was to determine the compatibility and stability of morphine and furosemide combined in solution at two different concentrations and stored in elastomeric infusors protected from light at 25ºC and 37ºC over a period of 15 days.

MATERIAL AND METHODS

Materials

Commercial morphine ampoules of 20 mg/mL (Morphine, Braun, Spain) and commercial furosemide ampoules of 20 mg/2 mL (Furosemide, Fresenius Kabi, Spain) were used. Sodium chloride 0.9% was obtained from Fresenius Kabi, Spain. HPLC-grade acetonitrile was obtained from Sigma-Aldrich. Other chemical and solvents were of analytical grade and obtained from Sigma-Aldrich, Germany. High purity water (resistivity 18.2 MΩ cm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout this work.

Drug mixtures

The doses of morphine and furosemide assayed in the study were chosen taking into consideration those more frequently used by the units of palliative care in our region. The doses assayed were 3.0 mg/mL-2.0 mg/mL and 1.0 mg/mL–0.6 mg/mL of morphine and furosemide respectively, which were prepared in 0.9% normal saline for injection and stored at two temperatures, 25°C and 37ºC each one, employing a bacteriological and culture oven with temperature and time regulation and digital reading, Selecta (INCUDIGIT 19L 2001246). Each of these four alternatives were prepared in triplicate in elastomeric infuser and protected from light and also in glass. From each mixture, five standards of different concentrations between 25 mg/mL and 160 mg/mL of admixture were prepared. The standards were divided into different

Table 1: Statistical evaluation of data for different stability studies

Morphine 3000 mg/L-Furosemide 2000 mg/L							
Mixture (mg/L)	Mean (mAU)	Standard deviation(mAU)	Variation co-efficient (%)	Minimum (mAU)	Maximum (mAU)	Confidence level (mAU)	
						Lower	Upper
25	401302	10328.2	2.5	384764	418392	388519	414085
50	786610	17395.2	2.2	747382	816979	773827	799393
75	1.16894E6	24395.0	2.1	1.13458E6	1.22736E6	1.15616E6	1.18172E6
100	1.52877E6	45284.0	2.9	1.42979E6	1.57785E6	1.51598E6	1.54155E6
125	1.96128E6	36051.1	1.8	1.87858E6	2.03426E6	1.9485E6	1.97406E6
Morphine 1000 mg/L- Furosemide 600 mg/L							
Mixture (mg/L)	Mean (mAU)	Standard deviation (mAU)	Variation co-efficient (%)	Minimum (mAU)	Maximum (mAU)	Confidence level (mAU)	
						Lower	Upper
32	544266	26103.9	4.7	505872	594437	534662	553870
64	972979	30031.7	3.0	920315	1.03607E6	963375	982583
96	1.4587E6	38701.4	2.6	1.33988E6	1.53624E6	1.44909E6	1.4683E6
128	1.92714E6	35780.5	1.8	1.86326E6	2.03565E6	1.91754E6	1.93674E6
160	2.44182E6	36412.1	1.4	2.35097E6	2.53176E6	2.43221E6	2.45142E6

Table 2: Concentrations obtained for the admixtures stored into infusors at 25ºC and 37ºC

[Admixture] ± SD* (mg/L) (3000 mg/L-2000 mg/L)							
25ºC				37ºC			
Day	Inf 1	Inf 2	Inf 3	Day	Inf 1	Inf 2	Inf 3
0	50	50	50	0	50	50	50
1	51.7 ± 0.5	53.6 ± 0.5	53.3 ± 0.7	1	51.1 ± 0.4	52.0 ± 0.2	50.3 ± 0.7
2	51.9 ± 0.3	52.3 ± 0.3	53.9 ± 0.3	2	47.3 ± 0.4	42.9 ± 0.5	45.2 ± 0.8
3	53.4 ± 0.3	50.7 ± 0.8	51.4 ± 0.4	3	45.9 ± 0.8	44.9 ± 1.2	45.1 ± 0.4
7	52.2 ± 0.3	45.9 ± 0.2	47.8 ± 0.4	7	43.1 ± 1.7	40.8 ± 0.4	42.6 ± 0.9
8	53.4 ± 0.6	47.9 ± 0.1	48.3 ± 0.3	8	-	38.2 ± 1.5	43.6 ± 0.9
10	52.3 ± 0.6	46.4 ± 1.5	46.1 ± 0.2	9	-	40.6 ± 0.6	44.7 ± 0.4
14	53.4 ± 0.6	43.7 ± 0.3	41.4 ± 0.5	-	-	-	-
15	52.1 ± 0.3	-	42.3 ± 0.4	-	-	-	-

*Mean ± standard deviation (n=3)

Table 3: Concentrations obtained for the admixtures stored into infusors at 25ºC and 37ºC

[Admixture] ± SD* (mg/L) (1000 mg/L-600 mg/L)							
25ºC				37ºC			
Day	Inf 1	Inf 2	Inf 3	Day	Inf 1	Inf 2	Inf 3
0	64	64	64	0	64	64	64
1	64.0 ± 1.2	63.4 ± 0.7	64.6 ± 0.8	1	63.4 ± 1.8	61.2 ± 0.3	62.6 ± 0.7
2	63.4 ± 0.9	61.4 ± 0.6	62.7 ± 0.9	2	56.1 ± 0.2	51.3 ± 0.4	53.0 ± 0.5
3	62.7 ± 2.1	62.1 ± 1.2	62.1 ± 0.7	3	50.0 ± 0.8	53.2 ± 0.7	52.3 ± 0.5
4	62.7 ± 1.3	61.4 ± 2.1	61.4 ± 1.3	4	49.8 ± 0.5	50.7 ± 0.3	53.1 ± 0.4
8	61.4 ± 1.6	61.4 ± 1.0	62.1 ± 1.6	7	39.2 ± 1.0	42.7 ± 0.2	47.7 ± 0.3
10	62.1 ± 2.2	60.8 ± 2.1	60.8 ± 2.3	8	33.5 ± 1.7	41.1 ± 0.5	52.5 ± 0.5
14	62.1 ± 1.4	62.1 ± 1.5	60.8 ± 1.5	9	-	37.5 ± 0.3	48.0 ± 0.2
15	62.7 ± 1.1	60.8 ± 1.4	61.4 ± 2.2	10	-	29.1 ± 0.8	36.6 ± 0.9

*Mean ± standard deviation (n=3)

aliquot parts, stored in eppendorf tubes and frozen until each analysis day. All the procedures were done under aseptic conditions and using sterile drug solutions.

Physical stability study

The physical stability of the samples was assessed by visual examination during all studied days for colour change and/or precipitation.

Chemical stability study

Mixtures concentrations were determined by a stability-indicating HPLC method. HPLC analysis was performed at room temperature (~25ºC) using a Shimadzu LC-6A pump equipped with Rheodine 7125 injection valve 20 µL, a Shimadzu SPD-6A spectrophotometric detector working at 235 nm. The signal from the detector was recorder and integrated with a chromatography data system Shimadzu C-R6A chromatopac; a LiChrospher® 100 C18 (5 µm) LiChroCART® 250-4 column was employed. The mobile phase consisted of acetonitrile: water (40:60, v/v) delivered at flow rate of 1.0 mL/min. The sample injection volume was 20 µL, and triplicate injections were performed for every sample. The initial concentration of mixture was defined as 100%, and subsequent sample concentrations were expressed as a percentage of the initial concentration. Stability of the mixture was defined as retention of at least 95% of the initial mixture concentration.

Forced degradation analysis

Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation path ways and degradation products of the drug substance.[12-13]

In this work, six different studies were carried out for this purpose over the mixture solution: acid, base, heat, UV light, hydrogen peroxide and sodium hypochlorite.

Compatibility and stability studies

The compatibility and stability studies were performed at 25 ± 0.5ºC and 37 ± 0.5ºC, and all drug mixtures were

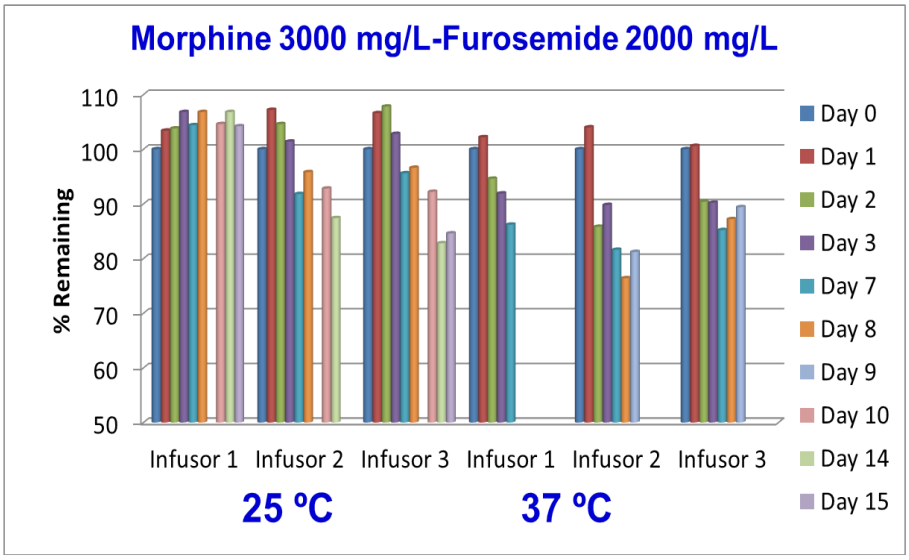

Figure 1: Percentages of morphine-furosemide mixtures (3000 mg/L-2000 mg/L) remaining at 25ºC and 37ºC

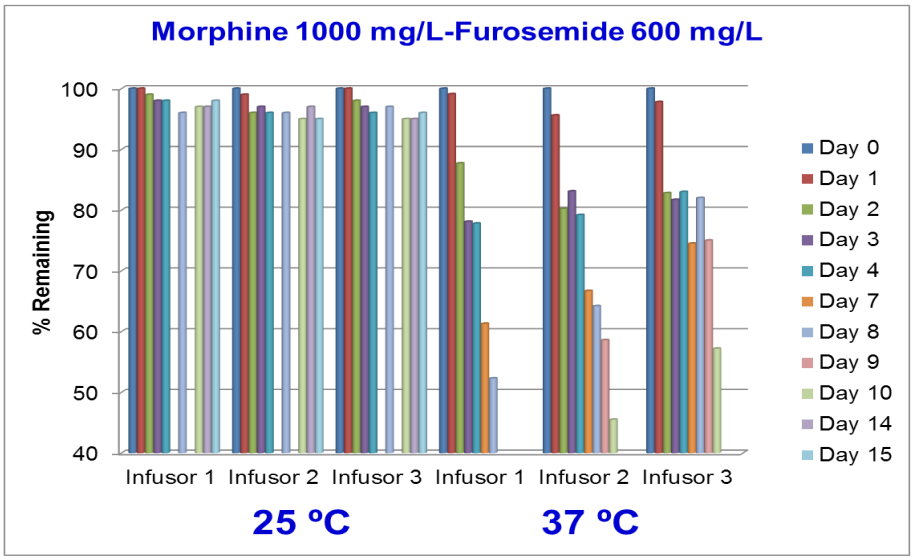

Figure 2: Percentages of morphine-furosemide mixtures (1000 mg/L-600 mg/L) remaining at 25ºC and 37ºC

protected from direct light exposure. All solutions were assayed in triplicate with four replicates in each case. At pre-determined times–that is 0, 1, 2, 3, 7, 8, 9, 10, 14, 15 days-the samples were examined for any development and/or change in colour. Also, the drug mixtures were examined for signs of precipitation or cloudiness (turbidity) and gas production under bright light against a dark background.

Table 4: Stability of admixtures

Morphine-Furosemide (mg/mL-mg/mL)	Days	
	25ºC	37ºC
3.0 mg/mL-2.0 mg/mL	8	2
1.0 mg/mL-0.6 mg/mL	30	2

RESULTS AND DISCUSSION

Accelerated degradation study

The subsequent studies were made over mixture solutions containing 40 mg/L of morphine and 24 mg/L of furosemide.

pH study

To aliquots of 500 µL of mixture were added different amounts of HCl or NaOH (0.1 M and 1 M) (100, 200, 300, 500 of both concentration).Additions of HCl or NaOH have not influence about the chromatographic signal. The area diminishes by effect dilution when the amount of degradant is higher and also the signal is constant with the time.

Heat study

Three samples of mixture solutions were heated at different temperatures (40ºC, 60ºC, 80ºC) during different times (from 5 to 60 minutes). No significant changes were observed in the chromatograms in all cases.

UV light

A mixture solution was subject to UV irradiation during several days. After one day under UV radiation, the signal of the admixture diminishes and also colour change is observed into glass.

Oxidants

To aliquots of 500 µl of mixture were added different amounts of NaClO 0.2 M and 2 M or H_2O_2 0.03%, 0.3 and 3% (100, 200, 300, 500 µl of each reagent and concentration). No effects were observed when the concentration of reagents were lower. The chromatographic signal increases and also stays constant with the time when 0.3% and 3% ofH_2O_2 were used.

Physical stability study

All solutions were initially clear and colourless and remained so for the duration of the study. Visible particles appear into the infusers at the same time decreased the concentration of the admixture stored into they.

Statistical evaluation of data

Calibration curves were linear over the concentration range used with good correlation coefficients. Statistical evaluations of data for different stability studies are presented in Table 1 for each mixture.

Chemical stability study

All the physically stable solutions were chromatographied. The experimental data were processed making use of the Statgraphics Centurion XVI program. The linearity of the method was evaluated at 5 concentration levels injected by quadruplicate varying from 25 mg/L to 160 mg/L. The standard calibration curves exhibited good linearity over the range of concentrations tested, with correlation coefficients greater than 0.999 in all cases. The concentrations obtained for each mixture at two studied temperatures (25ºC and 37ºC) are shown in tables 2 and 3. The percentages remaining corresponding to different mixtures are shown in figures 1 and 2.

CONCLUSION

This study was proven to be suitable for determining the stability and compatibility of morphine and furosemide mixtures in elastomeric infusors. It may be applied to stablish the stability of different samples prepared in NaCl 0.9 % and stored at two temperatures and can be used in palliative care (Table 4). It can be prepared in advance and stored at room temperature for at least 8 days, but the infusion with a system worn close to a patient that may reach a temperature closer to 37ºC cannot be longer than two days for both concentrations.

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SUMMARY

- Mixture of Morphine-Furosemide (3.0 mg/mL-2.0 mg/mL) is stable eight days at 25ºC and two day at 37ºC
- Mixture of Morphine-Furosemide (1.0 mg/mL-0.6 mg/mL) is stable thirty days at 25ºC and two day at 37ºC

About Authors

Espinosa Bosch Maria was resident in the pharmacy of the UniversitaryHospital Virgen del Rocio in Seville, Spain. During her training, she obtained a Bachelor's Degree in Health Applied Statistics at UniversityAutónoma of Barcelona. Actually she is working in the UGC Pharmacy in Regional Universitary Hospital of Málaga. Principal investigator of the project obtained in public call from the Junta of Andalucia, Spain for to study the compatibility and stability of different mixtures of drugs and its use in palliative care.

Sánchez Rojas Fuensanta and **Bosch Ojeda Catalina**, both professor of Analytical Chemistry in the University of Málaga, andthey teach in different degrees (Chemistry, Environment, Chemistry Engineer) and the same time, they are investigators in the study by HPLC of the stability of drugs mixtures in different condition of storage and concentrations. They have some review papers about the determinations of different drugs.

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