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Original Article

COMPARATIVE LC-MS STABILITY INDICATIND ASSAYS OF ONDANSETRON HYDROCHLORIDE/NALOXONE HYDROCHLORIDE AND METOCLOPRAMIDE HYDROCHLORIDE/NALOXONE HYDROCHLORIDE USED IN PALLIATIVE CARE

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ABSTRACT

Objectives: To compare the compatibility and chemical stability of ondansetron hydrochloride/naloxone hydrochloride and metoclopramide hydrochloride/naloxone hydrochloride admixtures used in palliative care units at three different storage conditions (4˚C, 22 ˚C and 37˚C) for 192 hours.

Methods: A high performance liquid chromatography-mass spectrometry (LC-MS) analytical method was established to investigate the chemical stability of the combinations.

Results: Metoclopramide hydrochloride and naloxone hydrochloride concentrations remain above 90% of their initial concentration under all storage conditions for 192 hours, while ondansetron hydrochloride remain stable at 4˚C and 22˚C but it losses up to 15.03% of its initial concentration when stored for 192 hours at 37 ˚C.

Conclusion: metoclopramide hydrochloride and naloxone hydrochloride admixture is more stable and preferred to ondansetron hydrochloride and naloxone hydrochloride admixture under all storage conditions.

Keywords: Palliative care-liquid chromatography-mass spectrometry-high performance liquid chromatography-ondansetron hydrochloride-metoclopramide hydrochloride-naloxone hydrochloride.

INTRODUCTION

The administration of painkillers, such as analgesics in palliative care of patients suffering from serious life-threatening diseases such as cancer is an essential part of pharmaceutical care in the hospitals. The main aim of palliative care is to prevent or treat the symptoms and side effects of the disease and its treatment [1]. Therefore, different drug combinations in a small volumes delivered to the patients by a syringe driver are used to overcome most of the annoying symptoms and side effects associated with fatal disease or its treatment.

Among the potential problems of mixing injections include degradation of the drug(s) and this may result in drugs precipitation/crystallization with potentially reduced drug efficacy. The greater the number of injections mixed together, the greater the possibilities of drug-drug interactions and changes in the physical nature of the drugs. Chemical stability and compatibility studies data are only available for a few of the subcutaneous and intravenous injection combinations used in palliative care; injection combinations are often used in practice without any prior assessment of the chemical or even physical stability of the admixture. In July 2009, the Medicines and Healthcare Products Regulatory Agency (MHRA, UK) published the outcome of its consultation regarding the legal position of practitioners mixing and administering medicines in palliative care together with the Commission on Human Medicines' recommendations for changes to medicines legislation.

The changes proposed apply not only to palliative care but to all clinical areas where the mixing of medicines is acceptable practice. The Commission's recommendations have been accepted by Ministers and will a) allow doctors and dentists to direct other to mix, b) allow non-medical prescribers to mix medicines themselves and direct others to mix, and c) allow nurse and pharmacist, independent prescribers, to prescribe unlicensed medicines for their patients on the same basis as doctors and supplementary prescribers [2]. As the MHRA intends to extend the types of practitioners authorized to be involved in the mixing of medicines and the situations where mixing of medicines is acceptable, there could well be an increase in the number of medicines that are mixed. This will certainly require an assessment of the physical and chemical stability of drugs when medicines are mixed. While many medicines are may be assumed to be stable under this situation, without investigation, the stability of such combinations should not be assumed.

Opioids are the first line choice for the palliative care provider to control moderate to severe pain associated with cancer patients [3]. However, opioids have serious aggravating side effects such as nausea, vomiting, respiratory depression, sedation and constipation [4-6]. Therefore, ondansetron hydrochloride, metoclopramide hydrochloride and naloxone hydrochloride are considered essential medicines in palliative care to reverse opioid's side effects. Ondansetron hydrochloride (Zofran) is a new serotonin subtype 3 ($5\text{-}HT_3$) receptor antagonist used to prevent or treat nausea and vomiting [7], whereas metoclopramide hydrochloride belongs to dopamine antagonist antiemetic class. In the other hand, naloxone hydrochloride is an opiate antagonist used for complete or partial reversal of respiratory depression induced by opioids [8]. Although metoclopramide hydrochloride has a serious side effects such as involuntary movement of the face, tongue, extremities and neuroleptic malignant syndrome, it is still prescribed as a powerful antiemetic to control nausea and vomiting despite of ondansetron has been reported in literature to be more effective with fewer side effects in prevention of postoperative nausea and vomiting [9]. In palliative care, although both subcutaneous injections of 4 mg/ml ondansetron hydrochloride [10, 11] and intravenous infusions of metoclopramide hydrochloride 2 mg/Kg [12] are recommended to control nausea and vomiting, 0.2 mg/ml of naloxone hydrochloride [13, 14] can be mixed with both formulations to manage respiratory depression occur due to the use of opioids to overcome moderate and severe pain. Although the admixtures considered to be compatible, physically and chemically stable at 4˚C [15] and 22˚C [16], admixtures physical and chemical stability weren't compared

and investigated at higher storage temperatures and hot climate. In the current study, a comparison between admixtures of ondansetron hydrochloride (0.4 mg/ml)/naloxone hydrochloride (0.2 mg/ml) and metoclopramide (0.2 mg/ml)/naloxone hydrochloride (0.2 mg/ml) in 0.9% sodium chloride were stored at 3 different temperatures (4, 22, 37 ^{0}C) for 8 days to indicate the more stable and compatible admixture to be used more safely in temperatures higher than 4˚C.

METHODS AND MATERIALS

Chemicals and materials

High performance liquid chromatography (HPLC) grade acetonitrile, ammonium acetate Analytical and acetic acid were obtained from Sigma Aldrich (Dor-set, UK). HPLC grade water was prepared ‘‘in house’’ with a MilliQ filter (Millipore, Watford, UK).

Ondansetron hydrochloride (Zofran) was available as 8 mg/4 ml ampoules (Glaxo Wellcome, Italy), metoclopramide hydrochloride powder was purchased from Sigma-aldrich (USA), naloxone hydrochloride was available as 0.4 mg/ml ampoules from HIKMA Pharmaceuticals (Amman, Jordan).

Three-part, polypropylene Luer-LokTM syringes (30 mL) were obtained from Becton Dickinson (Oxford, UK), and Helapet Combi-Caps from B. Braun (Melsungen, Germany).

Preparation of the combination studied

Stock solutions containing 1 mg/ml of both ondansetron hydrochloride/naloxone hydrochloride dehydrate dissolved in 0.9% sodium chloride solution and 1 mg/ml of both metoclopramide hydrochloride/naloxone hydrochloride dihydrate dissolved in 0.9% sodium chloride solution were prepared. Linearity of response around the nominal content in both injections was achieved with six concentrations ranging from 25 to 125 % diluted with 0.1% ammonium acetate in water (pH 4.5): acetonitrile (70:30 v/v).

Each admixture were prepared in duplicate and stored at 22˚C, 37˚C and 4˚C. Samples were analyzed at zero minutes, 24, 48, 96 and 192 hours.

pH measurement

The combinations were diluted X5 with HPLC grade water, and the pH of the diluted solution was measured with a pH meter that was calibrated with buffers at pH 4 and pH 7.

Instrumentation

The HPLC system (Waters 2690 Separation Module) used in this analytical method consisted of a Waters 600E multi solvent delivery system pump, a Waters Ultra WISP 715 auto-injector, and a Waters 996 diode-array detection system. Chromatographic separation was performed using Waters XTerra RP 18 (5 µm, 250 x 4.6 mm i. d) column

Chromatographic conditions

Mobile phase comprised of filtered and degassed 0.1% w/v ammonium acetate in water (pH 4.5) and acetonitrile in proportion of 70:30 v/v and pumped at a flow rate of 1 ml/min. Samples were analyzed at a wavelength of 240 nm and were injected at 10 µm injection volume. In LC-MS, the same conditions as were used in HPLC but at a flow rate of 2 ml/min. LC was directly attached to the ESI of triple quadrupole.

Degradation

One milliliter of each admixture was placed in a 4 mL vial to which 1 mL of 1 M HCl was added. Samples were heated to 90 ˚C for 90 min, then allowed to cool down for 15 min and analyzed by liquid chromatography–mass spectrometry (LC–MS).

LC-MS

Tandem MS was performed by a Waters Alliance 2695 Separations Module HPLC, equipped with a quaternary pump and an automatic interfaced to a Micromass Quattro micro API (triple quadrupole) mass spectrometer equipped with a Z-spray electrospray (ESI) ionization source was used. Nitrogen as drying, as well as nebulizing gas, was generated from pressurized air in a NG-7 nitrogen generator. The nebulizing gas flow was set to 50 L/h and the gas flow desolvation to 550 L/h.

The optimized values were: capillary voltages, 4.5 kV; extractor voltage, 2 V; source temperature; 100 ˚C; desolvation temperature, 400 ˚C; and multiplier; 650 V.

RESULTS

Samples of ondansetron hydrochloride/naloxone hydrochloride and metoclopramide hydrochloride/naloxone hydrochloride admixtures were stored at three storage conditions 4 ˚C, 22 ˚C and 37 ˚C and analyzed at 0 minutes, 4, 24, 48, 96, 192. fig. 1 shows the chromatogram obtained for ondansetron hydrochloride and naloxone hydrochloride combination after storage at 37 ˚C for 24 hours, while fig. 2 shows the chromatogram obtained for ondansetron hydrochloride and naloxone hydrochloride combination in the same storage conditions after 192 hours.

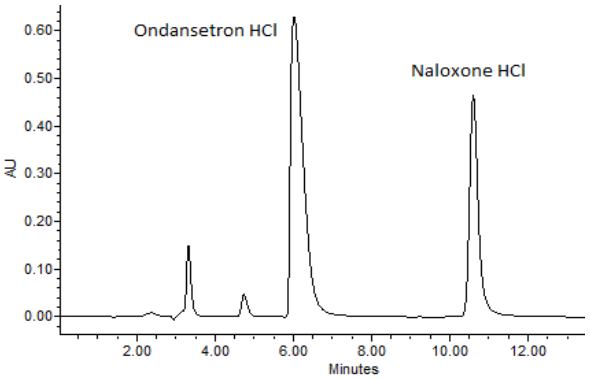

Fig. 1: High-performance liquid chromatography analysis of an injection combination containing ondansetron hydrochloride (0.4 mg/ml) and naloxone hydrochloride (0.2 mg/ml) in 0.9% water of injection (sodium chloride) after 24 hours storage at 37 ˚C

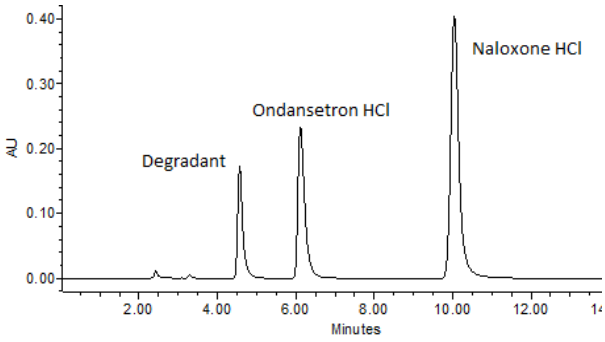

Fig. 2: High-performance liquid chromatography analysis of an injection combination containing ondansetron hydrochloride (0.4 mg/ml) and naloxone hydrochloride (0.2 mg/ml) in 0.9% water of injection (sodium chloride) after 8 days storage at 37 ˚C

Tables [1-6] show the set of data obtained for ondansetron hydrochloride/naloxone hydrochloride and metoclopramide hydrochloride/naloxone hydrochloride admixtures under fridge temperature (4 ˚C), room temperature (22 ˚C) and 37 ˚C.

Abbreviations

RSD: Relative standard deviation

Table 1: Stability data for admixture injection containing ondansetron hydrochloride (0.4 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at room temperature (22 ˚C)

	Ondansetron hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100. 0%	100.0%	0.0%
4	97.61%	97.45%	±0.1%	98.27%	98.26%	0.0%
24	97.25%	97.35%	±0.1%	98.09%	98.06%	0.0%
48	97.05%	97.23%	±0.1%	97.31%	97.76%	±0.3%
96	96.87%	96.76%	±0.1%	97.02%	97.01%	0.0%
192	95.88%	95.88%	0.0%	96.13%	96.00%	±0.1%

Table 2: Stability data for admixture injection containing ondansetron hydrochloride (0.4 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at room temperature (4 ˚C)

	Ondansetron hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100. 0%	100.0%	0.0%
4	98.52%	98.72%	±0.1%	97.32%	97.44%	±0.1%
24	98.28%	98.66%	±0.3%	97.28%	97.29%	0.0%
48	98.19%	97.92%	±0.2%	97.12%	96.73%	±0.3%
96	98.09%	97.28%	±0.6%	96.80%	96.35%	±0.3%
192	97.87%	97.17	±0.5%	96.61%	96.19	±0.3%

Table 3: Stability data for admixture injection containing ondansetron hydrochloride (0.4 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at room temperature (37 ˚C)

	Ondansetron hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100. 0%	100.0%	0.0%
4	97.39%	98.25%	±0.6%	97.93%	97.81%	±0.1%
24	93.35%	96.85%	±2.6%	94.51%	95.32%	±0.6%
48	90.62%	92.66%	±1.6%	93.30%	92.97%	±0.3%
96	88.33%	90.89%	±2.0%	91.85%	91.05%	±0.6%
192	84.97%	86.12%	±1.0%	90.05%	90.27%	±0.2%

Table 4: Stability data for admixture injection containing metoclopramide hydrochloride (0.2 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at room temperature (22^{0}C)

	Metoclopramide hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100% 0%	100.0%	0.0%
4	99.7%	99.5%	±0.1%	99.4%	98.35%	±0.8%
24	99.5%	99.5%	0.0%	99.4%	98.17%	±0.9%
48	98.89%	98.77%	±0.1%	97.95%	97.73%	±0.2%
96	98.33%	98.16%	±0.1%	95.72%	97.58%	±1.4%
192	97.61%	97.35%	±0.2%	95.12%	96.90%	±1.3%

Table 5: Stability data for admixture injection containing metoclopramide hydrochloride (0.2 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at (4 ˚C)

	Metoclopramide hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100. 0%	100.0%	0.0%
4	99.98%	99.86%	±0.1%	99.63%	99.65%	±0.01%
24	99.00%	99.53%	±0.4%	98.69%	99.21%	±0.4%
48	97.61%	97.95%	±0.3%	96.07%	96.88%	±0.6%
96	97.44%	97.22%	±0.1%	96.05%	96.61%	±0.4%
192	95.96%	96.88%	±0.7%	95.71%	96.28%	±0.4%

Table 6: Stability data for admixture injection containing metoclopramide hydrochloride (0.2 mg/ml) and naloxone hydrochloride (0.2 mg/ml) under light exposure at (37 ˚C)

	Ondansetron hydrochloride % remaining			Naloxone hydrochloride% remaining		
Time (h)	**Syr.1**	**Syr.2**	**RSD%**	**Syr.1**	**Syr.2**	**RSD%**
0	100.0%	100.0%	0.0%	100. 0%	100.0%	0.0%
4	99.93%	98.72%	±0.03%	99.89%	99.42%	±0.5%
24	99.76%	97.91%	±0.4%	99.27%	98.88%	±0.7%
48	98.71%	95.72%	±0.2%	99.01%	97.99%	±1.7%
96	97.03%	95.49%	±0.3%	97.42%	96.20%	±0.5%
192	95.81%	95.38%	±0.2%	96.06%	95.86%	±0.4%

Degradant peak eluted at around 4.3 minutes was identified by using Tandem MS as shown in fig. 3.

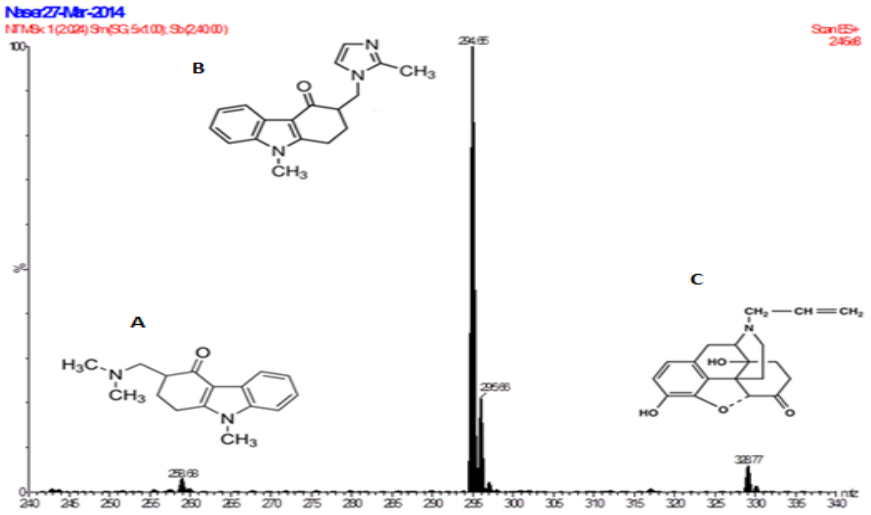

Fig. 3: Full scan ESI mass spectrum of A-3[(dimethylamino) methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol-4-one B-Ondansetron C-Naloxone

DISCUSSION

Although ondansetron hydrochloride and metoclopramide hydrochloride were recommended to be administered in 4 mg/ml and 2 mg/Kg respectively, concentrations were reduced to 0.4 mg/ml and 0.2 mg/ml correspondingly to be within the instrument range. The calibration curves for ondansetron hydrochloride/ naloxone hydrochloride and metoclopramide hydrochloride/ naloxone hydrochloride were linear over the range between 25% and 200% of the stated content for ondansetron hydrochloride, metoclopramide hydrochloride and naloxone hydrochloride in 0.9% sodium chloride infusion. Moreover, the stability indicating high performance liquid chromatography assay method for the indication and quantification of metoclopramide was very simple and capable to produce resolved and symmetrical peaks with reasonable retention time compared with the literature reported assays [17] which used an ion-pairing agent and complex mobile phase composition. Method precision was tested by preparing 6 samples of each admixture and analyzed using the chromatographic conditions. The relative standard deviation of peak area obtained for ondansetron hydrochloride, metoclopramide hydrochloride and naloxone hydrochloride were±0.6%,±0.4% and±0.9%, respectively. Under the examined conditions, one degradant peak is clearly eluted before ondansetron hydrochloride peak. Relatively small amounts of degradant were noticeable for ondansetron hydrochloride under room temperature (22˚C) and fridge temperature (4 ˚C) for 8 days.

While after 8 days storage at 37 ˚C, ondansetron hydrochloride was prone to degradation up to 15.02%. In contrast, metoclopramide hydrochloride and naloxone remain above 90% of their initial concentration under all storage conditions for 8 days. Degradant peak is formed after 4 days at all storage conditions, the area of the degradant peak increased as the temperature increased. The identification of degradant was performed using Tandem MS and it was due to the formation of ondansetron related compound A (3[(dimethylamino) methyl]-1,2,3,9-tetrahydro-9-methyl-4H-carbazol -4-one) as stated in European pharmacopeia [18]. On the other hand, metoclopramide hydrochloride and naloxone hydrochloride admixture formulation remain stable for 8 days under all storage conditions and there was not any detectable traces of degradants. However metoclopramide hydrochloride/naloxone hydrochloride admixture showed no color change during all storage conditions for 8 days, visual inspection of ondansetron hydrochloride/naloxone hydrochloride admixture showed that the appearance changed with time going from a clear, colorless solution when prepared to a brown-colored solution, this became noticeable after 6 days. Apart from the dis coloration of ondansetron hydrochloride/naloxone hydrochloride admixture from visual inspection, there was no evidence of particulate formation and the pH of ondansetron hydrochloride/naloxone hydrochloride was reduced over 192 hours, while metoclopramide hydrochloride/naloxone hydro chloride admixture pH remained stable over 192 h for both admixtures. In addition, degradation study showed that ondansetron hydrochloride was degraded in 1 M hydrochloric acid to form ondansetron related compound A, while metoclopramide hydrochloride remain stable.

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CONFLICTS OF INTERESTS

Author has no conflicts of interest to declare

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