Chemical and microbiological stability studies of an aqueous solution of pravastatin sodium salt for drug therapy of the dysphagic patients

Giulia Bonacucina,¹ Serena Logrippo,¹ Marco Cespi,¹ Roberta Ganzetti,² Luca Casettari,³ Matteo Sestili,² Diego Romano Perinelli,¹ Massimo Ricciutelli,¹ Antonella Marziali,⁴ Carlo Polidori,¹ Giovanni Filippo Palmieri¹

¹School of Pharmacy, University of Camerino, Camerino, Italy

²Hospital Pharmacy, Italian National Research Centers on Aging (I.N.R.C.A.), Ancona, Italy

³Department of Biomolecular Sciences, University of Urbino, Urbino, Italy

⁴Hospital Microbiological Laboratory, Italian National Research Centers on Aging (I.N.R.C.A.), Ancona, Italy

Correspondence to

Giulia Bonacucina, School of Pharmacy, University of Camerino, via S. Agostino, 1 Camerino 62032, Italy; giulia.bonacucina@unicam.it

Received 27 August 2015

Revised 13 January 2016

Accepted 26 January 2016

Published Online First

23 February 2016

ABSTRACT

Objective This study is aimed to improve dysphagic patient compliance under therapy with cholesterol-lowering drugs. Patients suffering severe dysphagia, who do not feed independently, receive enteral nutrition through feeding tube and they need alternative oral route also for the administration of pharmacological therapy. This research deals with the development and stability (chemical and microbiological) of an aqueous solution of pravastatin sodium salt that will be administered orally directly in the feeding tube starting from commercial tablets. Tablets formulation is the only pharmaceutical dosage form available on the market for this type of drug.

Methods Pravastatin sodium salt tablets are dissolved in a preserved sodium bicarbonate solution at the final concentration of 4 mg/mL. Samples are stored in two different conditions until 60 days. The samples are prepared for high-performance liquid chromatography analysis coupled to a diode array detector (HPLC-DAD), microbiological analysis and pH measurements.

Results The chemical stability of the solution performed with HPLC-DAD analysis shows peaks' overlapping, which are characteristic of pravastatin, and correspondence of the concentration of the active ingredient in the solution. The detected values are analysed by one-way analysis of variance showing no statistically significant differences. Microbiological analyses proved that there is not microbial growth. By considering the dilution factor applied, it was possible to express the result as <10 CFU/mL in the two different culture media.

Conclusion This study demonstrated the possibility to reformulate pravastatin tablets as liquid pharmaceutical formulation for enteral administration with the aim of improving drug therapy in dysphagic patients.

INTRODUCTION

Swallowing is a complex function involving several nerves and muscles that act in reflected and synchronised way after an initial volunteer swallowing. Dysphagia is defined as difficulty swallowing; more specifically, a sensation causing one to perceive an impairment in the passage of food from mouth to stomach.¹ The main consequences of dysphagia are different and can imply a social aspect as patient's isolation due to difficulty in swallowing or medical aspect such as respiratory problems, due to penetration of bolus in the airway, suction by

tracheobronchial tree, choking and *ab-ingestis* pneumonia.^{2–4} Another implication regards the nutritional problem, which means reduction of spontaneous feeding with progressive weight loss until protein-calorie malnutrition, dehydration, electrolyte unbalances, reduction of immune defences that might require enteral nutrition (EN) administered through a feeding tube (nose-gastric, nose-duodenal, nose-jejunal or orogastric) or Percutaneous Endoscopy Gastrostomy.^{5–8}

There are various causes of dysphagia and both geriatric and paediatric patients are affected by it. This manuscript mainly focuses on geriatrics, but the studied preparation can be addressed to paediatric population after correct dosage revision. In fact, it is possible to distinguish between 'presbifagia', mainly due to patient's age, and dysphagia, the term used when pathological conditions are also present. Dysphagia is common in patients having pathologies which involve the central nervous system (CNS), as for example, neurodegenerative pathologies, such as Alzheimer's or Parkinson's diseases, or in presence of strokes due to damages in specific brain areas. Specifically, it has been reported that swallowing difficulties are found in 60%–80% of patients with moderate to severe cognitive impairment⁹; moreover, dysphagia affects 40%–80% of patients in the first week post stroke¹⁰ and persists in the 3%–17% of the cases after 1 month from vascular event.¹¹

Swallowing deficits can be related also to pharmacological or radiotherapeutic treatments. For example, molecules having antipsychotic activity, with their extrapyramidal side effects, reduce pharyngeal muscle tone interfering with dopaminergic and adrenergic receptors causing dysphagia.¹² In addition, radiotherapy, alone or together with chemotherapy, can affect swallowing ability when the oropharyngeal area is treated.¹

Finally, dysphagia could be also a consequence of head, neck and thoracic area's surgery, due to the potential damaging of the nervous, muscular and cartilaginous structures involved in swallowing process.¹

When patients are unable to swallow common solid dosage forms, different formulations need to be used and often an alternative route is chosen.

A common practice is to crush tablets or open capsules prior to the administration^{5 13 14} which requires a manipulation of original pharmaceutical formulation that makes it necessary to consider

CrossMark

To cite: Bonacucina G, Logrippo S, Cespi M, et al. *Eur J Hosp Pharm* 2016;**23**:288–293.

different issues such decrease of drug efficacy, increase in toxicity, problems of instability, lowering of palatability and loss of invaluable amount of drugs. Besides, when a solid oral dosage form is administered through a different way with respect to that authorised by pharmaceutical company, the manipulation and administration responsibility is early on the prescriber and then on the person who administered.¹⁵ For example, when an oral liquid formulation is not available, it is common practice to open the capsule or crush the tablet and then mix it with food or beverages to allow administration through a feeding tube.

In this paper, chemical-analytical and microbiological stability of an aqueous solution of pravastatin sodium salt starting from tablets has been investigated with the aim to administer the solution orally by using syringe directly in feeding tube. The work deals with stability studies on pravastatin sodium salt, HMG-CoA (3-hydroxy-3-methyl-glutaryl-Co-enzymeA) reductase inhibitor belonging to the family generically called statin-series compounds. Physicians prescribe this molecule for treating primary disorder such as hypercholesterolaemia or hyperlipidaemia in addition to well-balanced diet, primary and secondary prevention of cardiovascular diseases and post solid-organ transplant. Tablet is the only pharmaceutical dosage form for this type of drug and the formulation of an aqueous solution is performed to improve compliance and pharmacological therapy in dysphagic patients treated with cholesterol-lowering drugs.

METHODS

Materials

- ▶ Pravastatin sodium salt 20 mg tablets (Pensa Pharma);
- ▶ Sodium bicarbonate 8.4% p/v solution (S.A.L.F. Laboratorio Farmacologico);
- ▶ Methyl p-hydroxy benzoate sodium (Sharon Laboratories);
- ▶ Propyl p-hydroxy benzoate sodium (Clariant);
- ▶ Sterile polypropylene (PP) microtubes 2 mL (Sarstedt AG & Co);
- ▶ PP syringes 30 mL plus needle 19G (Rays);
- ▶ Culture medium Columbia Agar +5% sheep blood-COS (Columbia sheep) in Petri plates (BioMerieux);
- ▶ Culture medium SABOURAUD gentamicin chloramphenicol—SGC2 in Petri plates (BioMerieux).

Samples preparation

Aqueous solution of pravastatin sodium salt¹⁶ was prepared according to Good Manufacturing Practice (GMP Ph. Eur. VIII Ed.). Briefly, methyl p-hydroxy benzoate sodium, propyl p-hydroxy benzoate sodium and then, 20 mg tablets of pravastatin sodium salt were added to a sodium bicarbonate 8.4% solution under magnetic stirring¹⁷ up to a final pravastatin concentration of 4 mg/mL. The need to use basic diluent was linked to assure a final pH solution in a specific range of 7–10, according to the suggestion detailed in the last-mentioned reference.

The final aqueous solution formulation contains: (1) pravastatin sodium salt 20 mg tablets, 20 units; (2) methyl p-hydroxy benzoate sodium, 0.150 g; (3) propyl p-hydroxy benzoate sodium, 0.050 g and (4) sodium bicarbonate 8.4% solution q.s. 100 mL.

Chromatography analysis

Chemical stability of the aqueous solution containing pravastatin sodium salt 4 mg/mL was evaluated using a high-performance liquid chromatography system coupled to a diode array detector (HPLC-DAD) method supported by ChemStation for LC 3D

system.^{18–21} The ultraviolet–visible DAD was set at 210–220–230 nm (Agilent ChemStation for LC 3D System). The active compound pravastatin sodium salt was monitored and quantified at 230 nm (wavelength of maximum absorption). Pravastatin sodium salt was separated isocratically on a C18 reverse phase analytical column (Varian, 5 μ m, 4.6×250 mm). The mobile phase was prepared from monobasic potassium phosphate and phosphate acid in HPLC grade water. The 0.02 M buffer solution was adjusted to pH 3.0 with phosphate acid and mixed with methanol (HPLC-GOLD-Ultragradient Carlo Erba Reagenti) (60:40v/v). The flow rate was set at 1 mL/min. The injection volume was 1 μ L and the column temperature was controlled at 30°C. HPLC-DAD studies were performed using an Agilent technology 1100 series, made of an auto-sampler and a binary solvent pump.

In order to validate analytical HPLC method, the calibration curve is obtained by plotting the peak area against a solution of pravastatin sodium salt at different concentrations (1, 2, 4, 8 mg/mL), with a good correlation $R^2 \geq 0.999$. Limit of detection and limit of quantification values are 0.001 and 0.003 mg/mL, respectively.

With the calibration curve it was possible to check the active compound dosage in the used tablets and to verify the declared amount by the manufacture.

According to section 2.9.6 of the Italian Pharmacopoeia (FUI), ‘Uniformity of content of the pharmaceutical forms in single dose’,²² the preparation respected the essay if the declared amount is in the range of 85%–115% compared with mean value. The analysed tablets fall within the required range.

A total of 51 samples listed above are divided into:

- ▶ three samples frozen at –20°C at time 0;
- ▶ 24 samples stored in the refrigerator (2°C–8°C) without light exposure;
- ▶ 24 samples stored at room temperature (22°C–25°C) without light exposure.

Temperature, samples’ weight and microtubes’ appearance were carefully controlled during 60 days.

Three samples among that stored in refrigerator and three among that stored at room temperature are frozen at –20°C at the days 1, 5, 8, 14, 21, 30, 46, 60 from the beginning.

In order to check any variations in the aqueous solution’s composition, not caused by the tablet inside the preparation, 17 reference samples of 1 mL are prepared. They have the same composition of solution with the exception of the tablets.

Also the references were divided into:

- ▶ one sample frozen at –20°C at time 0;
- ▶ eight samples stored in the refrigerator (2°C–8°C) without light exposure;
- ▶ eight samples stored at room temperature (22°C–25°C) without light exposure.

At days 1, 5, 8, 14, 21, 30, 46, 60, the corresponding samples were frozen at –20°C and then, chromatographic analyses were performed only for samples at the days 0, 14, 30, 60.

The number of analysis was reduced because of a good stability of the active ingredient in the solution as confirmed by the samples at 60 days and second, also for those at 14 and 30 days. Before analysis, samples’ weight was controlled for verifying any variations. Then, every microtubes were centrifuged at 13 300 rpm for 5 min and the content was filtered with 0.22 μ m filter and syringed in a vial.

Duplicate HPLC determinations are performed on each analysis time and on each storage condition in order to have variability within the sample and between samples. The results were enough for a suitable statistical interpretation of the obtained outcomes.

At days 0 and 60, the reference samples were tested in order to underline any potential variations not due to the presence of the tablets in the solution.

Stability analysis

An HPLC evaluation for physical and chemical stabilities was evaluated for 60 days at two storage conditions: room temperature (22°C–25°C) and refrigerated (2°C–8°C). Thus, 68 samples were prepared from original aqueous solution of pravastatin sodium salt and packed in PP sterile microtubes 2 mL. In particular, 51 samples of 1 mL for chromatographic analysis and 17 samples of 1.5 mL for pH evaluation were prepared.

Every sample was labelled with a specific name to be identified and the weight of each one was checked to monitor any variations (table 1).

The visual examination of solution colour and the pH values measured through a digital pHmeter (Denver Instrument—BASIC) on days 0, 14, 30, 60 were assessed as a further confirmation of the stability of the liquid solution.

Microbiological analysis

Four PP syringes, each containing 20 mL of solution, were prepared and protected from light, then, two syringes were preserved at room temperature and two in refrigerator.

Table 1 Samples' weight of pravastatin sodium salt aqueous solution

Sample's name	Weight after preparation mean±SD	Weight before freezing mean±SD	Weight after unfreezing mean±SD
0.1	2.59±0.0004	2.60±0.0002	2.61±0.0003
0.2	2.61±0.0001	2.61±0.0003	2.62±0.0004
0.3	2.61±0.0002	2.59±0.0001	2.60±0.0003
Reference 0	2.62±0.0001	2.61±0.0003	2.67±0.0002
14.1 R.T.	2.63±0.0005	2.62±0.0002	2.63±0.0004
14.2 R.T.	2.65±0.0005	2.65±0.0002	2.65±0.0002
14.3 R.T.	2.61±0.0007	2.61±0.0001	2.61±0.0002
14.1 REF.	2.62±0.0003	2.62±0.0000	2.62±0.00006
14.2 REF.	2.62±0.0003	2.62±0.0000	2.62±0.0002
14.3 REF.	2.64±0.0008	2.64±0.0000	2.64±0.0004
30.1 R.T.	2.65±0.0002	2.65±0.0003	2.66±0.0002
30.2 R.T.	2.63±0.0006	2.63±0.0002	2.63±0.0002
30.3 R.T.	2.64±0.00006	2.64±0.0002	2.62±0.0005
30.1 REF.	2.63±0.0005	2.63±0.0002	2.63±0.0001
30.2 REF.	2.62±0.0004	2.65±0.0003	2.63±0.0001
30.3 REF.	2.63±0.0008	2.63±0.0003	2.64±0.0001
60.1 R.T.	2.62±0.0002	2.62±0.0006	2.62±0.0001
60.2 R.T.	2.63±0.0008	2.63±0.0002	2.63±0.0002
60.3 R.T.	2.66±0.0005	2.66±0.0006	2.64±0.0004
Reference 60 R.T.	2.64±0.0005	2.66±0.0003	2.65±0.0001
60.1 REF.	2.67±0.0003	2.67±0.0002	2.67±0.0001
60.2 REF.	2.64±0.0001	2.64±0.0002	2.64±0.0001
60.3 REF.	2.63±0.0003	2.64±0.0002	2.62±0.0002
Reference 60 REF.	2.65±0.0004	2.63±0.0005	2.65±0.0003

X.1 R.T., day X sample 1 room temperature; X.2 R.T., day X sample 2 room temperature; X.3 R.T., day X sample 3 room temperature; X.1 REF., day X sample 1 refrigerator; X.2 REF., day X sample 2 refrigerator; X.3 REF., day X sample 3 refrigerator; Reference X R.T., reference sample day X at room temperature; Reference X REF., reference sample day X in refrigerator.

According to section 5.14 of FUI, 'with microbiological quality of pharmaceutical preparations and substances for pharmaceutical non sterile use',²² orally used preparations have to respect these criteria:

1. Total aerobic microbial count (TAMC): maximum acceptable 200 CFU/mL;
2. Total combined yeasts and molds count (TYMC): maximum acceptable 20 CFU/mL.

About 1 mL of pravastatin sodium salt aqueous solution taken at days 0, 1, 5, 8, 14, 21, 30, 46, 60 from the preparation date, from each previous prepared syringe, was sown in duplicate on Petri plates by seeding method on surface. For the TAMC, the used culture medium was Columbia Agar +5% sheep blood—COS and the plates were incubated, upside down, in a thermostat at 30°C–35°C (34°C) for 3 days.

Instead, for the TYMC, the used culture medium was SABOURAUD gentamicin chloramphenicol—SGC2 and the plates were incubated, upside down, in a thermostat at 20°C–25°C (24°C) for 5 days. The seeding procedures were performed under laminar flow hood. Before the seeding, each sample was diluted 1:10 with sterile water. After incubation period, any microbial colonies grown were estimated in each type of medium culture plates and then, average values were registered. Because the analysis was carried out on diluted samples, in order to express results, it was necessary to multiply the obtained CFU values by 10. Instead, the microbial growth was registered as <10 CFU/mL (applied dilution factor).

In order to validate the microbiological method, specific American Type Culture Collection (ATCC) strains were used: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231. The only difference in the seeding procedures for the specific microbial strains used was the addition of Tween 80 (1% diluent sterile water) to the diluted sample to neutralise the parabens put in the formulated aqueous solution.

Moreover, to confirm method and in order to carry out 'Microorganisms Recovery Test', positive and negative controls were performed in duplicate. For the positive check, 100 µL suspension of the specific strain ATCC were added to 900 µL diluent and the total was sown on the plates COS and SGC2 with the same seeding procedures and for the same incubation period. Instead, for the negative one, the sterility of diluent (water) and culture media and the operating condition were monitored.

Statistical analysis

Significant differences among the means of the obtained concentrations were determined through one-way analysis of variance using Minitab program (V15.1.0.0). A p-value less than 0.05 was considered statistically significant. p-Values for aqueous solution are 0.232 at room temperature and 0.117 in the refrigerator.

RESULTS

Chromatography analysis

The identification of pravastatin sodium salt, as active compound, in the solution was assessed by comparison with the retention time of the pravastatin sodium salt standard (retention time 6.94 min). A further confirmation of this qualitative analysis was achieved by comparison with related ultraviolet spectra obtained by DAD detector.

The chromatograms gave a great overlapping of the peaks that identify pravastatin sodium salt in all analysis time (0, 14, 30, 60 days from the preparation) and in both storage

Figure 1 High-performance liquid chromatography analysis coupled to a diode array detector chromatograms showing the aqueous solution of pravastatin sodium salt at time 0–14–30–60 days stored (A) at room temperature and (B) in the refrigerator.

conditions (figure 1A,B). Average peak area was calculated and it was possible to deduce relative concentrations of active compound in the solution through the calibration curve. These values were compared with the amount of pravastatin sodium salt declared by the company. The mean concentration of the active ingredient in the solution was 4.36 (0.076) mg/mL that was equivalent of 21.79 (0.38) mg as dosage of pravastatin sodium salt in each tablet. These values were included in the required range in FUI. The results demonstrated that the molecule was stable because there are no significant changes in the obtained data (figure 2). The analysis performed on the

reference samples at days 0 and 60 showed that there were no variations due to the presence of the tablets in the reference solution. The temperature was not a significant parameter because the two different detected storage conditions did not affect the analysis results. This aspect can be important for the management of the drug therapy both in the hospital and at home.

Microbiological analysis

The microbial tests were carried out on the aqueous samples at every established time in the programme work and in both storage conditions, in order to have a strict monitoring of any

Figure 2 Quantification of active compound pravastatin sodium salt in the aqueous solution stored at room temperature and in the refrigerator.

microbiological growth. After the incubation periods, 3 CFU/mL of *Corynebacterium jeikeium* were detected in two samples of Columbia Agar +5% sheep blood plates used for TAMC evaluation and 3 CFU/mL of *Penicillium* spp were detected in one sample of SABOURAUD gentamicin chloramphenicol plates used for TYMC evaluation. These results demonstrated that the micro-organisms found in the liquid preparation most likely were the result of a contamination during the preparation steps or during analysis although GMP was observed in both cases.

Considering the limited number of CFU/mL in both culture media, it was possible to express the result as <10 CFU/mL (applied dilution factor). Moreover, a complete microbiological stability of pravastatin sodium salt solution 4 mg/mL can be declared, both when it was held at room temperature and when it was preserved in the refrigerator.

The suitability of the analytical method was assessed verifying that the mean value obtained for each micro-organism test in presence of product (ATCC strain) was compared with the mean value obtained in the positive control ('Microorganism Recovery Test').

Recovery percentage for each micro-organism used was calculated according to this formula:

Table 3 Recovery test of aqueous solution of pravastatin sodium salt stored in the refrigerator

Recovery test of aqueous solution—refrigerator	Mean value obtained in presence of product CFU/plates	Mean value in positive control CFU/plates	Recovery %
<i>Staphylococcus aureus</i> ATCC 6358 (Agar COS)	22	38	57.8
<i>Pseudomonas aeruginosa</i> ATCC 9027 (Agar COS)	66	45	146.6
<i>Escherichia coli</i> ATCC 8739 (Agar COS)	24	28	85.7
<i>Candida albicans</i> ATCC 10231 (Agar SGC2)	18	16	112.5

ATCC, American Type Culture Collection.

Recovery %

$$= \frac{(\text{mean value obtained in presence of product})}{(\text{mean value in positive control})}$$

This percentage has to be in the range of 50%–200% (factor of 2) and this criterion has to be respected by the four specific micro-organisms selected for this study and by all batches of products used in the analytical method's validation (tables 2 and 3).

pH stability

The pH measurements of the aqueous solution showed a substantial stability of the preparation and respected the stability range of the active ingredient (table 4). These results confirmed the stability of new aqueous pravastatin formulation.

DISCUSSION

This study offers an alternative treatment for the management of drug therapy in dysphagic patient who cannot take solid oral dosage forms such as tablets or capsules. The topic about improving the administration of drugs in patients with swallowing difficulties is discussed every day in the hospitals, in the nursing homes and at home among relatives who have to manage the intake of therapy by the member of their family.

Therefore, the development of this liquid pharmaceutical form, safe and exact in the dosage, can help nurses and caregivers in the administration of medication in patients who cannot swallow. The analytical HPLC-DAD method used allows an exact quantification of each injected sample in the instrument and a right amount of drug administered to patient. Microbiological tests assure a high grade of microbial stability until 60 days. This period allows organising the management of the galenic preparations of the aqueous solution according to the necessities of the clinical pharmacy.

Table 2 Recovery test of aqueous solution of pravastatin sodium salt stored at room temperature

Recovery test of aqueous solution—room temperature	Mean value obtained in presence of product CFU/plates	Mean value in positive control CFU/plates	Recovery %
<i>Staphylococcus aureus</i> ATCC 6358 (Agar COS)	42	38	110.5
<i>Pseudomonas aeruginosa</i> ATCC 9027 (Agar COS)	55	45	122.2
<i>Escherichia coli</i> ATCC 8739 (Agar COS)	29	28	103.5
<i>Candida albicans</i> ATCC 10231 (Agar SGC2)	19	16	118.7

ATCC, American Type Culture Collection.

Table 4 pH values of aqueous solution of pravastatin sodium salt

Aqueous solution samples (day)	Room temperature pH mean±SD	Refrigerator pH mean±SD
0	8.52±0.026	
14	8.57±0.020	8.53±0.052
30	8.63±0.089	8.58±0.050
60	8.72±0.040	8.60±0.260

CONCLUSION

The stability study performed through chemical-analytical and microbiological analyses and the pH measurements show that the active compound is stable for all the considered period of 60 days. Moreover, it is possible to administer the aqueous solution of pravastatin sodium salt to dysphagic patients in order to improve their drug therapy even when they are in EN.

What this paper adds

What is already known on this subject

- Patients with swallowing difficulty require the correct management of drug therapy, especially because solid oral dosage forms (eg, tablets and capsules) may cause choking or can remain in patient's mouth or oesophagus.
- In this contest, those who administer medication have to manipulate the original pharmaceutical formulation, despite this procedure being not safe and can cause a lot of risks for prescriber and patient.
- Currently, some practices such as chopping and mixing tablets with water or food are followed, but it is necessary to consider the introduction of a medication review to improve the management of drug therapy in dysphagic patient.

What this study adds

- This study demonstrated the possibility to formulate and administer an aqueous solution of pravastatin sodium salt to dysphagic patients in order to improve their drug therapy even when they are in enteral nutrition.
- This new preparation can be administered through feeding tube in order to obtain exact dosage of the drug, avoiding risks for patient and medico-legal implications for those who administer or prescribe the therapy.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- 1 Stegemann S, Gosch M, Breikreutz J. Swallowing dysfunction and dysphagia is an unrecognized challenge for oral drug therapy. *Int J Pharm* 2012;430:197–206.
- 2 Cockeram AW. Canadian association of gastroenterology practice guidelines: evaluation of dysphagia. *Can J Gastroenterol* 1998;12:409–13.
- 3 Australian and New Zealand Society for Geriatric Medicine. Australian and New Zealand Society for Geriatric Medicine. Position Statement—dysphagia and aspiration in older people. *Australas J Ageing* 2011;30:98–103.
- 4 Fine MJ, Auble TE, Yealy DM, et al. A prediction rule to identify low risk patients with community-acquired pneumonia. *N Engl J Med* 1997;336:243–50.
- 5 Williams NT. Medication administration through enteral feeding tubes. *Am J Health Syst Pharm* 2008;65:2347–57.
- 6 Estoup M. Approaches and Limitations of Medication Delivery in Patients With Enteral Feeding Tubes. *Crit Care Nurse* 1994;14:68–72, 77–9; quiz 80–1.
- 7 Gora ML, Tschampel MM, Visconti JA. Considerations of drug therapy in patients receiving enteral nutrition. *Nutr Clin Pract* 1989;4:105–10.
- 8 Bowman C. Administration of drugs to patients with swallowing difficulties. *J Malta College Pharmacy Practice* 2007;12:42–5.
- 9 Schiele JT, Quinzler R, Klimm HD, et al. Difficulties swallowing solid oral dosage forms in a general practice population: prevalence, causes, and relationship to dosage forms. *Eur J Clin Pharmacol* 2013;69:937–48.
- 10 Martino R, Foley N, Bhogal S, et al. Dysphagia after stroke: Incidence, diagnosis, and pulmonary complications. *Stroke* 2005;36:2756–63.
- 11 Perry L, Love CP. Screening for dysphagia and aspiration in acute stroke: a systematic review. *Dysphagia* 2001;16:7–18.
- 12 Goodman & Gilman. The pharmacological basis of Therapeutics. XI edition. Laurence L. Brunton, John S. Lazo, Keith L. Parker.
- 13 Wright D. Medication administration in nursing homes. *Nurs Standard* 2002;16:33–8.
- 14 Barnes L, Cheek J, Nation RL, et al. Making sure the residents get their tablets: medication administration in care homes for older people. *J Adv Nurs* 2006;56:190–9.
- 15 Griffith R, Griffiths H, Jordan S. Administration of medicines part 1: the law and nursing. *Nurs Standard* 2003;18:47–53.
- 16 Neuvonen PJ, Backman JT, Niemi M. Pharmacokinetic comparison of the potential Over-the-Counter statins Simvastatin, Lovastatin, Fluvastatin and Pravastatin. *Clin Pharmacokinet* 2008;47:463–74.
- 17 Ishibashi M, Mitsuru E, Yasushi M. Gel preparation for oral administration. U.S. Patent Application 12/010,741.
- 18 Otter K, Mignat C. Determination of pravastatin in human plasma by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl* 1998;708:235–41.
- 19 Piecha M, Sarakha M, Trebše P, et al. Stability studies of cholesterol lowering statin drugs in aqueous samples using HPLC and LC–MS. *Environ Chem Lett* 2010;8:185–91.
- 20 Campos-Lara M, Pinto-Almazan R, Oropeza MV, et al. Optimization of a Pravastatin quantification method using HPLC with ultraviolet detection in human serum for monitoring dyslipidemic patients. *J Liq Chromatogr R T* 2008;31:667–74.
- 21 Bauer S, Mwinyi J, Stoeckle A, et al. Quantification of pravastatin in human plasma and urine after solid phase extraction using high performance liquid chromatography with ultraviolet detection. *J Chromatogr B* 2005;818:257–62.
- 22 *Farmacopea Ufficiale della Repubblica Italiana XII edizione*, Ministero della Salute. Roma: Istituto Poligrafico dello Stato, 2008.