Chapter 8

Presentations

<table>
<thead>
<tr>
<th>International - 04</th>
<th>National - 01</th>
<th>Total – 05</th>
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1. Separation and Characterization of forced degradation product of tolteridone tartarate by LC-MS/MS. Analytical Research Forum, Organized by Royal Society of Chemistry, Great Britain in Durham University, UK, July 2012. Received full International Travel Grant including Registration Fees from Department of Science and Technology (DST), Govt. of India.


Publications


5. Development and Validation of Packed Column Supercritical Fluid Chromatographic Technique for Quantification of Chlorzoxazone, Paracetamol and Aceclofenac in their Individual and Combined Dosage Forms. *Journal of Chromatographic Science 2012; 50:769–774.* (Impact Factor 0.88)


Various approaches for impurity profiling of pharmaceuticals - An overview

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Received on: 11-02-2011; Revised on: 16-03-2011; Accepted on: 21-04-2011

ABSTRACT

Tremendous attention is given for the impurity profiling of pharmaceutical products in present time. Impurities can endanger the human body by affecting quality, safety and efficacy of the product. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Due to the noticeable impact of impurities on quality of pharmaceuticals, impurity control in pharmaceutical products is a primary goal of drug development. To assure the quality of drugs, impurities must be monitored carefully. Stringent international regulatory requirements have been in place for several years as outlined in USFDA, Canadian Drug and Health Agency, International Conference on Harmonization. In this review, a description of different types of impurities and their origin in relation to ICH guidelines are presented. The article further throws light on different methodological aspects of impurity profiling such as NMR, MS, TLC, HPLC, HPTLC, GC, CE and other hyphenated techniques like, LC-MS, GC-MS, LC-NMR, CE-MS and ICP-MS which are routinely used for monitoring impurities.

Keywords: Impurities, ICH guidelines, Instrumental techniques, Hyphenated techniques.

1. INTRODUCTION

Dictionary meaning of impurity is something that is impure or makes another thing impure. So, impurity in short way can be defined as, any material that affects the purity of the material of interest, i.e., an active pharmaceutical ingredient (API) or drug substance. Impurity can also be defined as 'any substance coexisting with the original drug substance, such as starting material or intermediates from reaction or that is formed, due to any chemical interaction or by products from side reaction'.[1]

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (API's) or develop during formulation or upon aging of both API and formulations.[2]

Many potential impurities arise during the synthesis of API. Sources of impurity includes, starting materials, intermediates, reagents, solvents, catalysts and reaction by products. These potential impurities should be identified to find out their origin and how to minimize these impurities at initial level itself. The amount of these impurities present in drug substance (API) will determine the safety of drug product. Therefore identification, quantification, qualification and control of impurities are now crucial part of drug development.[3]

The presence of some impurities may not have harmful impact on drug quality if they have therapeutic effect that is similar to or greater than the drug substance itself. However, a drug substance can be considered as compromised with respect to purity if it contains an impurity with superior pharmacological or toxicological properties.[4]

The various regulatory authorities emphasizing on the impurity profiling includes ICH, USFDA, Canadian Drug and Health Agency and Guidelines given by department of health and ageing therapeutic goods administration of Australian government.[5] The different official compendia, such as British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and European Pharmacopoeia (EP) are incorporating limits to restrict levels of impurities present in API as well as in formulations.[6] Table 1 shows the thresholds of impurities specified by ICH guidelines.[7]

Table 1: Thresholds for Impurities as per ICH guidelines.

<table>
<thead>
<tr>
<th>Maximum daily Dose</th>
<th>Reporting threshold</th>
<th>Identification threshold</th>
<th>Qualification threshold</th>
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<tr>
<td>≤ 2 g/day</td>
<td>0.05%</td>
<td>0.10% or 1.0 mg per day</td>
<td>0.13% or 1.0 mg per day in take ( whichever is lower) or 0.05%</td>
</tr>
<tr>
<td>&gt; 2 g/day</td>
<td>0.03%</td>
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2. CLASSIFICATION OF IMPUTITIES

Pharmaceuticals can be divided into two major areas active pharmaceutical ingredients (APIs) which is also referred as drug substance (DS) and drug product (DP) which is also called as finished pharmaceutical product (FPP).

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Imurities observed in API may arise during synthetic route and they can be of different types such as organic impurities, organic volatile impurities or inorganic impurities. During DP development, impurities may observed either as degradation products because of inherent instability of drug substance, or as a result of incompatibility of drug with added excipients, or from interaction of drug with primary packaging materials used.[8] Figure 1 shows various sources of pharmaceutical impurities.

So impurities found in pharmaceuticals are basically of two types as follows:
1. Impurities associated with active pharmaceutical ingredients (API).
2. Impurities that are formed during formulation and or with ageing or that are related to the formulated forms.

In general according to ICH guidelines, identification of impurities below 0.1% level is not considered necessary unless the potential impurities are expected to be unusually potent or toxic.[8] According to the ICH guidelines, impurities related to API’s are classified in to the following main categories:[8-10]

A. Organic impurities
B. Inorganic impurities
C. Residual solvents

A. Organic impurities:
Organic impurities may arise in drug substance during the manufacturing process (synthesis) or storage of the drug substances. They may be identified or unidentified, volatile or non-volatile. Following are the sources of organic impurities,[8-10]

a) Starting material(s): This is the most common source of impurity found in every API unless a proper care is taken in every step involved in its manufacturing. Although the end products are always washed with solvents, there are always chances of having the residual untreated starting materials remaining unless the manufacturer is very careful about them.[8-10]

b) Intermediates: These are compounds formed during synthesis of the drug substance.[8-10]

[1] Figures

Figure 1. Tree diagram for different sources of impurities.
Development and Validation of RP-HPLC, UV-Spectrometric and Spectrophotometric Method for Estimation of Tapentadol Hydrochloride in Bulk and in Laboratory Sample of Tablet Dosage Form

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ABSTRACT

Three novel, simple, accurate and rapid methods have been developed and validated for estimation of tapentadol hydrochloride in bulk and in laboratory tablet sample. In RP-HPLC method, elution was achieved in isocratic mode using combination of 50mM phosphate buffer pH 3.62 and acetonitrile in ratio of 70:30 (% v/v) with 0.1% triethylamine and using HiQ Sil C8 column having specification, 250 x 4.6 mm and 5µm particle size. The flow rate was 1 ml/min and detection was done at 285 nm. UV-Spectrophotometric estimation of tapentadol was carried out at 272 nm. Third method consists of quantification of tapentadol using Folin-Ciocalteu reagent in presence of 20% sodium carbonate solution. The blue colour chromogen formed is measured at wavelength of maximum absorption 750 nm for tapentadol against reagent blank. All three developed methods were validated according to ICH guidelines. Furthermore assay results obtained by three methods were compared statistically as well.

Keywords: Tapentadol Hydrochloride, RP – HPLC, UV- Spectrophotometry, Folin-Ciocalteu reagent.

INTRODUCTION

Agonists of the µ-opioid receptor are being used conventionally for the treatment of moderate to severe pain. Tapentadol hydrochloride (TAP) is novel centrally-acting oral analgesic.

Chemically it is 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2methylpropyl] phenol monohydrochloride. It is not official in any pharmacopoeia. [1-3]

Up till now only few methods are reported for estimation of TAP which includes, estimation of tapentadol and its metabolite N-desmethyl tapentadol in urine and oral fluids by using ultra pressure liquid chromatography with tandem mass detection (LC-MS/MS).[4] Furthermore one study is reported which discusses about determination of four stereoisomers of TAP by X-ray crystal structure analysis.[5]

Literature survey revealed that no method has been reported for estimation of TAP in bulk as well as in formulation. Hence it was endeavored to develop various analytical methods like, RP-HPLC and UV- Spectrophotometric methods for estimation of TAP in bulk drug. So far marketed formulation of TAP is not available in Indian market. Hence a laboratory sample of tablet dosage form was developed to check applicability of developed method.
Development and Validation of Novel Spectrofluorimetric Method for Estimation of Tapentadol Hydrochloride in Bulk and in Laboratory Sample of Tablet Dosage Form

Sherikar O D1, Mehta P J1*

Abstracts: Novel, sensitive and accurate spectrofluorimetric method has been developed and validated for estimation of tapentadol hydrochloride in bulk and in laboratory tablet dosage form. The method is based on measurement of native fluorescence of tapentadol in distilled water at 298 nm after its excitation at 273 nm. The fluorescence intensity-concentration plot was linear in the concentration range of 1- 10 μg/ml with good correlation coefficient 0.9990. The developed method was successfully applied for determination of tapentadol hydrochloride in laboratory sample of tablet dosage form. Furthermore developed method was successfully validated as per ICH guidelines in terms of, linearity (1 - 10 μg/ml), repeatability (RSD 0.39 %), precision (intra-day variation, RSD, 0.17 to 0.63 % and inter-day variation, RSD, 0.34 to 0.63 %) and accuracy (99.44 to 99.62 %). The limit of detection and quantification was found to be 0.011 and 0.034 μg/ml respectively. The developed method proved to be simple, economic and precise. Therefore proposed method can be employed for the routine quality assessment of the tapentadol hydrochloride in bulk as well as in pharmaceutical dosage forms.

INTRODUCTION
Activation of the μ-opioid receptor the important alternative, which is commonly used for the treatment of moderate to severe pain. These are very useful in case of acute pain. Tapentadol hydrochloride (TAP) is novel centrally-acting synthetic analgesic. Chemically it is 3-[1(R, 2R)-3-(dimethyl amino)-1-ethyl-2methyl[propyl] phenol monohydrochloride. TAP is not official in any pharmacopoeia.1-3

Up till now only few methods have been reported for estimation of TAP which includes, estimation of TAP and its metabolite N-desmethyl tapentadol in urine and oral fluids by using ultra pressure liquid chromatography with tandem mass detection (LC-MS/MS). 4-5 In addition another reported study discusses about four stereoisomers of TAP. Furthermore the crystal and molecular structures of four stereoisomers of tapentadol hydrochloride have been determined by X-ray crystal structure analysis.6 Recently UV-Spectrophotometric 7 and RP-HPLC 8-10 methods have been reported for estimation of TAP.

Literature survey revealed that so far no study has been reported for estimation of TAP in bulk as well as in formulation by spectrofluorimetric technique. Hence it was endeavored to develop sensitive, accurate, and precise spectrofluorimetric method by using native fluorescence of TAP for estimation of TAP in bulk drug. When study was carried out marketed formulation of TAP was not available in Indian market. Hence a laboratory sample of tablet dosage form was developed to check applicability of developed method.

MATERIALS AND METHODS
Instrumentation
Spectrofluorimeter, FP 6500 with single quartz cell of 1 cm path length (Jasco, Japan) was used for fluorescence measurement. Spectra manager software was used for the data acquisition and data collection. All weighing was done on analytical balance (Model CX 220, Citizen India Ltd).

Materials
Working standard of tapentadol hydrochloride 99.9 % pure was used. Methanol AR grade was purchased from Rankem (Mumbai, India). Double distilled water used throughout study.

Experimental
Preparation of Standard Stock Solution
An accurately weighed quantity of 50 mg TAP was transferred into 50 ml volumetric flask. About 25 ml of methanol was added and sonicated to dissolve. The solution was cooled at room temperature and made up to volume with methanol to get final concentration of 1000 μg/ml.

Preparation of Working Standard Solution
TAP working standard solution was prepared by diluting standard stock solution (5.0 ml) to 50 ml with double distilled water (diluent) to produce required concentration (100 μg /ml).

Spectrofluorimetric Detection
The standard solution of TAP was scanned over the range of 220 nm to 400 nm wavelengths for selection of excitation wavelength in excitation mode. It showed highest florescence intensity at 273 nm (Figure 1). Therefore excitation wavelength 273 nm was selected to find out emission wavelength. The standard solution of TAP was scanned over the range of 220 nm to 400 nm wavelengths for selection of emission wavelength by selecting 273 nm as an excitation wavelength in emission mode. It showed highest florescence intensity at 298 nm (Figure 2). So, emission wavelength of 298 nm was selected for measurement of fluorescence intensity of TAP. Wavelength search in software also shows excitation and emission wavelength of 273 nm and 298 nm respectively (Figure 3).

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Comprehensive Assessment of Degradation Behavior of Aspirin and Atorvastatin Singly and in Combination by Using a Validated RP-HPLC Method

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Published:  December 11th 2012    Received:  October 18th 2012
Accepted:  December 11th 2012

This article is available from: http://dx.doi.org/10.3797/scipharm.1210-19

Abstract

A fixed-dose combination of atorvastatin and aspirin is widely used for the treatment of myocardial infarction. The present work describes a comprehensive study of the stress degradation behavior of atorvastatin and aspirin alone as well as in combination of 1:1 and 1:7.5 ratios, respectively, as per ICH guidelines. The degradation products of aspirin as well as atorvastatin were successfully separated by a developed simple, selective, and precise stability-indicating reversed-phase HPLC method. Chromatographic separation was achieved on the Phenomenex Luna analytical column, 150 mm x 4.6 mm, 5µm. The mobile phase consisted of 0.1% glacial acetic acid in water and acetonitrile in the ratio of 50:50 v/v at a flow rate of 1.0 ml/min. UV detection was performed at 246 nm. The extent of degradation was significantly influenced when both of the drugs were present in combination. Stress degradation behavior of atorvastatin was highly influenced by aspirin under acid hydrolysis, thermal degradation, and oxidative stress conditions. Similarly, the stress degradation behavior of aspirin was affected by atorvastatin especially under neutral hydrolysis, thermal degradation, and oxidative stress conditions. Additionally, the combination ratio of aspirin and atorvastatin also influenced the percentage degradation of each other. A mixture of aspirin and atorvastatin was also analyzed after a one-month stability study at 40 °C and 75% RH. All the results indicate chemical incompatibility of both aspirin and atorvastatin if present in combination.
Development and Validation of Packed Column Supercritical Fluid Chromatographic Technique for Quantification of Chlorzoxazone, Paracetamol and Aceclofenac in their Individual and Combined Dosage Forms

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Received 4 October 2011

A reproducible, rapid and sensitive method has been developed for the assay of chlorzoxazone (CHL), paracetamol (PCM) and aceclofenac (ACE) in their combined solid dosage forms using packed-column supercritical fluid chromatography (SFC). The analytes were resolved by elution with supercritical carbon dioxide doped with 15% v/v of modifier concentration on chromatographic parameters like retention time, retention factor, resolution, asymmetry and theoretical plates. Modifier concentration proved to be the most effective means for changing both retention and selectivity. The developed method was validated as per International Conference on Harmonization guidelines. The developed SFC method was compared with a reported high-performance liquid chromatography method for the estimation of CHL, PCM and ACE using Student t-test. With respect to the speed and use of organic solvents, SFC was found to be superior and eco-friendly. The developed SFC method was successfully used for the assay of different marketed formulations containing CHL, PCM and ACE individually and in combination.

Introduction

Supercritical fluid chromatography (SFC) is fast and environmentally friendly, especially when compared to high-performance liquid chromatography (HPLC). It is more cost-efficient and user friendly, with high throughput and short run time, and better resolution and faster analysis time than liquid chromatography (LC) and gas chromatography (GC) methods. In the recent era of science and technology, there has been a push for environmentally friendly or greener processes for the production and estimation of pharmaceutical compounds. The SFC technique minimizes the use of large amounts of organic solvents. Due to higher diffusion rates and low viscosity, SFC provides a 3–5-time increase in the speed of analysis and a decrease in cost of analysis by saving organic solvents. SFC is developing as an eco-friendly, green chromatographic technique. Thus, an attempt is made to develop a simple, eco-friendly analytical method for the estimation of widely used pharmaceutical drugs individually and in their combinations.

The combination of chlorzoxazone (CHL), paracetamol (PCM) and aceclofenac (ACE) is widely used in the treatment of musculoskeletal disorders (MSD) (Figure 1). Chemically, CHL is 5-chlorobenzoxazol-2(3H)-one, a centrally acting skeletal muscle relaxant with sedative properties. PCM, chemically \(N\)-(4-hydroxyphenyl) acetamide, a para-aminophenol derivative, has analgesic and antipyretic properties and weak anti-inflammatory activity. ACE, which is chemically 2-(2,6-dichloroanilino) phenylacetoxycetic acid, a phenylacetice acid derivative, is a non-steroidal anti-inflammatory drug (NSAID). It is possible that combination therapy of CHL, PCM and ACE provides relief from muscle and joint pain (1–4).

A literature review reveals that various methods like UV-Visible spectroscopy (5, 6), HPLC (7–12) and high-performance thin-layer chromatography (HPTLC) (13) have been used for the simultaneous analysis of CHL, PCM and ACE in their combined dosage forms. However, the UV-Visible spectroscopic method is not selective and techniques like HPLC and HPTLC require large volumes of organic solvents. Hence, the present study was undertaken to develop a simple, eco-friendly SFC method for simultaneous estimation of CHL, PCM and ACE in their individual and combined dosage forms.

Experimental

Chemicals and reagents

Carbon dioxide (99.9% pure) was obtained from BOC (Mumbai, India). PCM and HPLC-grade methanol were obtained from S.D. Fine Chemicals Mumbai, India. CHL and ACE were gifted by INTAS Pharmaceuticals (Ahmedabad, India).

Marketed formulations were procured from a local market. The following formulations were used for the study: three tablet formulations containing CHL, PCM and ACE (HIFENAC MR, Intas Pharmaceuticals, India; ACECLO MR, Aristo Pharma, India; RALIWIIZ MR, Wisdom Pharma, India); three tablet formulations containing CHL and PCM (DUODIL, Solvay Pharma, India); one tablet formulation containing CHL and PCM (AROFF PLUS, Unichem Pharma, India); and three tablet formulations containing CHL, PCM and ACE individually (PARAFON DSC, Jonson and Johnson, India; CALPOL, Glaxsmithkline, India; HIFENAC, Intas Pharmaceuticals, India).

Instrumentation

A Jasco-900 series SFC (Japan Spectroscopic Co.; Hachioji, Tokyo, Japan) was employed for the present study. It was...
Concurrent Estimation of Amlodipine Besylate, Hydrochlorothiazide and Valsartan by RP-HPLC, HPTLC and UV–Spectrophotometry

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Received 25 July 2012; accepted 20 November 2012

Accurate, sensitive and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC), high-performance thin-layer chromatography (HPTLC) and ultraviolet (UV) spectrophotometric methods were developed for the concurrent estimation of amlodipine besylate (AMLO), hydrochlorothiazide (HCTZ) and valsartan (VALS) in bulk and combined tablet dosage forms. For the RP-HPLC method, separation was achieved on a C18 column using potassium dihydrogen orthophosphate buffer (50 mM, pH 3.7) with 0.2% triethylamine as the modifier and acetonitrile in the ratio of 56:44 (v/v) as the mobile phase. Quantification was achieved using a photodiode array detector at 232 nm over a concentration range of 2–25 µg/mL for AMLO, 5–45 µg/mL for HCTZ and 20–150 µg/mL for VALS. For the HPTLC method, the drugs were separated by using ethyl acetate–methanol–toluene–ammonia (7.5:3:2:0.8, v/v/v/v) as the mobile phase. Quantification was achieved using UV detection at 242 nm over a concentration range of 100–600 ng/spot for AMLO, 150–900 ng/spot for HCTZ and 1,200–3,200 ng/spot for VALS. The UV–spectrophotometric simultaneous equation method was based on the measurement of absorbance at three wavelengths; i.e., at 237.6 nm (λ\text{max} of AMLO), 270.2 nm (λ\text{max} of HCTZ) and 249.2 nm (λ\text{max} of VALS) in methanol. Quantification was achieved over the concentration range of 2–20 µg/mL for AMLO, 5–25 µg/mL for HCTZ and 10–50 µg/mL for VALS. All methods were validated according to International Conference on Harmonization guidelines and successfully applied to marketed pharmaceutical formulations. Additionally, the three methods were compared statistically by an analysis of variance test, which revealed no significant difference between the proposed methods with respect to accuracy and precision.

Introduction

Amlodipine besylate (AMLO) is a calcium channel blocker that is commonly used in the treatment of hypertension and angina. Chemically, it is \(\text{RS}-3\text{-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulfonate} \) (Figure 1A) (1). Various analytical methods have been reported for the estimation of AMLO, alone or in combination with other antihypertensive agents in pharmaceutical formulations. These include ultraviolet (UV) spectroscopy (2–4), high-performance liquid chromatography (HPLC) (5–8), high-performance thin-layer chromatography (HPTLC) (9–10), liquid chromatography–mass spectrometry (LC–MS) (11) and LC–tandem mass spectrometry (MS–MS) (12).

Valsartan (VALS) is a potent specific angiotensin II receptor blocker that is a widely used antihypertensive agent. Chemically, it is \(\text{N-(1-oxopentyl)-N-[[2'-\text{OH-tetrazol-5-yl}] [1,1'-biphenyl]-4-yl] methyl]-L-valine} \) (Figure 1C) (13). Literature has revealed different methods for the quantification of VALS, alone and in combination with other antihypertensive drugs, such as HPLC (14–15), LC–MS (16–18), capillary electrophoresis (20) and simultaneous UV spectrophotometric methods (21–22), and methods in plasma (19).

Hydrochlorothiazide (HCTZ) is a thiazide diuretic used for treatment of high blood pressure. Chemically, it is \(2H-1,2,4\)-benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide \) (Figure 1B) (23). Many analytical methods have been reported for the quantification of HCTZ, alone or in combination with other drugs, which include spectroscopic and chromatographic methods (24–29). All three drugs can be found in the United States Pharmacopeia (USP) (30). AMLO and HCTZ are official drugs in both the Indian Pharmacopoeia (IP) (31) and the British Pharmacopoeia (BP) (32).

A literature survey has revealed several methods for the estimation of AMLO, VALS and HCTZ, individually or in combination with other drugs. No method has been reported for the simultaneous estimation of AMLO, VALS and HCTZ in their combined dosage forms by simultaneous equation using UV spectrophotometry. No HPTLC methods have reported for the simultaneous estimation of these three drugs in their combined dosage form.

Two RP-HPLC methods have been reported for this combination. The first method incorporates 55% of organic eluent and the second method utilizes approximately 70% of organic eluent. The present study reports a new RP-HPLC method that uses only 44% of organic eluent. In addition, the retention time of VALS in the proposed method is 10.15 min. With this higher retention time, stability studies for VALS in the presence of AMLO and HCTZ can be conducted. Because degradation products are mostly more polar than the parent compound, they will elute out before the parent drug. In such cases, the proposed method is advantageous, with higher retention time of VALS. Therefore, an attempt was made to develop accurate, precise and sensitive methods using simultaneous equation UV spectrophotometry and HPTLC, plus an alternative RP-HPLC method, for the concurrent estimation of AMLO, VALS and HCTZ in bulk and in their combined dosage form.

Experimental

Chemicals and reagents

Standard drug samples of AMLO, VALS and HCTZ were gifted by Torrent Research Centre (Ahmedabad, India). A marketed